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Natural succession of microalgal communities during the cold-water season and the impact of increased solar irradiance on sea ice algae

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CONTRIBUTIONS

	I	II	III
Original idea	SE, JMR	SE, JMR	JP, JMR
Study design and sampling	SE, JMR, RA	SE, AV, JMR, JP, MM, RA	SE, AV, JMR, JP, MM, RA
DNA extractions	JB, MM	-	-
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Data analysis	SE, JB, JMR, MM	SE, JMR, JP	SE, AV, JP, RS
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NATURAL SUCCESSION OF MICROALGAL COMMUNITIES DURING THE COLD-WATER SEASON AND THE IMPACT OF INCREASED SOLAR IRRADIANCE ON SEA ICE ALGAE

SARA ENBERG

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The winter season in polar and sub-polar areas is commonly described as a dormant phase for microalgae, especially in the water column, although the sea ice is a habitat for diverse and active communities. However, these areas are subject to changing environmental conditions due to global warming and changes in ultraviolet radiation. Sea-ice extent and ice thickness are additionally decreasing. Microalgae are primary producers in the ocean, a food source for consumers, and thus, temporal and spatial studies of microalgal communities are required to understand succession in these ecosystems and to be able to predict future changes. Ice cover in the Baltic Sea in Northern Europe forms and melts annually. The length of the ice-covered season and the extent of the ice cover vary between years, and depend on air temperature and other weather conditions during the winter.

This thesis describes the natural succession of microalgal communities in the water column and sea ice during the cold-water winter season, with a long-lasting ice-covered period. Results show that the cold-water season is dynamic with various microalgal communities and their variable photosynthetic activity in the water column and sea ice. Although microalgal biomass is relatively low throughout the cold-water season, especially in the water column, natural succession during the cold-water season can be divided into five different groups based on microalgal community composition. Similar to the water column, algal succession in the sea ice begins with low biomass and domination of various flagellates. Thereafter the biomass in the ice increases and the community shifts to being diatom and dinoflagellate dominated. In late spring, sea ice algae communities differ between the ice layers from the top to the bottom ice. The highest biomass in the bottom ice layer (dominated by colonial pennate diatoms), due to a better growth environment (e.g. more space, nutrient replenishment from the under-ice water) compared to the upper layers of the ice, where light intensity may additionally be too high and inhibit optimal microalgal growth. Although high microalgal biomass and photosynthetic activity occurs in the spring ice, the relative contribution of sea ice algae to the total areal primary production (ice and pelagic production) remains low (estimation 10 %, Haecky and Andersson 1999) due to thinness of the ice layer (< 1 m), which is characteristic to the Baltic Sea. However, microalgal biomass in the ice, especially during February and March, is higher (20–580 mg C m^{-3}) compared to the water column (10–20 mg C m^{-3}) during the ice-covered season. In addition, the contribution of released

dominant sea ice algae to the water column community is not significant, and the spring bloom community is largely formed by pelagic species of the early open-water season. Changing environmental conditions, especially increasing temperature, potentially lead to a shorter ice-covered season, smaller ice-covered area, thinner ice cover and less snow on the ice, which increases both photosynthetic active radiation (PAR) and ultraviolet radiation (UVR) in the sea ice. This thesis also describes the effect of enhanced solar irradiance on the microalgal community at various ice depths. The effects of solar radiation on sea ice algae have usually been studied in manipulation experiments focusing only on the bottom ice, where the light environment is different. Less photons are present in the bottom ice, and the quality of light is distinctly different compared to the upper layers depending on ice and snow cover thickness on top of the ice. We studied the effects of enhanced solar irradiance (including PAR and UVR) on the microalgal community at various depths of the spring ice during a three-week experimental *in situ* study. Results show that the largest effect of enhanced solar irradiance occurs in the top 10-cm layer of ice, but even beneath this layer the diminishing amount of irradiance and change in light quality increase the photosynthetic activity and change the community composition of the sea ice algae. Exposure to ultraviolet radiation also increases the concentration of mycosporine-like amino acids (MAA) and the variety of MAA compounds in the sea ice algae. Global warming is likely to result in thinner ice and could thus lead to changes in sea ice algae community structure in the Baltic Sea area, i.e. changes in the sea-ice pennate diatom community. The altered community and population dynamics in the sea ice may decrease the productivity and change the functioning of the system in the sea ice-pelagic coupling and bentho-pelagic coupling.

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1. CONTENTS

1. INTRODUCTION

1.1. Seasonal microalgal succession in the Baltic Sea

In temperate and sub-polar latitudes, e.g. the Baltic Sea in Northern Europe, the year is characterized by four calendar-based seasons (spring, summer, fall and winter) resulting from the Earth's axial tilt causing a great change in day length and altitude of the Sun during the year. These seasons also greatly affect both terrestrial and aquatic environments. Both macroalgae (e.g. Fong and Zedler 1993, Fricke et al. 2008, Deregibus et al. 2016) and phytoplankton (e.g. Ramberg 1979, Drake et al. 2010, Sommer et al. 2012) production in aquatic environments depend on the amount of solar irradiance. Phytoplankton succession in the Baltic Sea is well studied, especially during the productive, well-illuminated phase with high biomass, from the spring bloom to the summer and fall blooms (Kivi et al. 1993, Wasmund et al. 1998, Wasmund and Uhlig 2003, Gasiūnaitė et al. 2005, Suikkanen et al. 2007). However, much less is known of phytoplankton succession during the cold-water season with less solar irradiance. This information is necessary if we are to understand succession throughout the entire year and be able to predict future changes caused by climate change. In the following paragraphs the microalgal succession in the Baltic Sea is presented starting from the well-studied seasons (spring and summer) proceeding to the less-studied fall and wintertime succession in the water column and sea ice.

The spring bloom is a seasonal event characteristic to temperate and sub-polar areas. The spring bloom in the Baltic Sea typically occurs throughout the spring, which in the Northern Hemisphere lasts from the beginning of March to the end of May, but the onset of the spring bloom differs throughout the Baltic Sea. Spring bloom in the southern Baltic Sea usually begins at the beginning of March (Wasmund et al. 1998, Wasmund and Uhlig 2003, Fleming and Kaitala 2006), while it commences in the middle of March in the Gulf of Finland (Wasmund and Uhlig 2003, Fleming and Kaitala 2006, Lips et al. 2014) and at the beginning of April in the northern Baltic Sea (Andersson et al. 1996, Haecky et al. 1998). However, the onset of the spring bloom has shifted, beginning earlier compared to the 1980s and 1990s (Wasmund and Uhlig 2003, Fleming and Kaitala 2006). The spring bloom community is dominated by diatoms *Pauliella taeniata* (usually in the literature with its previous name *Achnanthes taeniata*), *Skeletonema* col., *Melosira* spp., *Thalassiosira* spp. and *Chaetoceros* spp. (Kuosa et al. 1997, Haecky et al. 1998, Höglander et al. 2004). The abundance and biomass of dinoflagellates increase later during the spring bloom, and the dinoflagellate community is dominated by *Peridiniella catenata* and *Woloszynskia halophila*/*Scrippsiella hangoei* (Wasmund et al. 1998, Höglander et al. 2004). In addition, diatoms dominate the spring bloom community composition after severe ice winters, but winters with a shorter ice-covered season result in spring blooms dominated by dinoflagellates (Wasmund et al. 1998, Klais et al. 2011).

Warmer weather and longer day length are characteristics of the summer season in temperate and sub-polar latitudes. Summer in the Northern Hemisphere extends from the beginning of June to the end of August based on the higher mean air temperature and the Gregorian calendar. The phytoplankton biomass in the northern Baltic Sea remains

low at the beginning of summer due to the low nutrient concentration, and the inorganic nitrogen concentration in particular is low after the spring bloom. At the beginning of summer, the phytoplankton community is dominated by green algae *Monoraphidium contortum*, small flagellates (including cryptophytes and haptophytes) and small dinoflagellates (including *Heterocapsa rotundata*, *Gymnodiniales* spp.) (Andersson et al. 1996, Balode et al. 1998). Cyanobacteria blooms are present during summer, however, nitrogen-fixing cyanobacteria form extensive blooms in mid-summer (mid-June, July) under calm weather conditions and warm waters. The most abundant cyanobacteria are filamentous species *Aphanizomenon* sp., *Dolichospermum* sp. (in the literature also with its previous name *Anabaena* sp.) and *Nodularia spumigena* (Andersson et al. 1996, Gasiūnaitė et al. 2005). The dinoflagellates *Heterocapsa triquetra*, *Dinophysis norvegica* and *Dinophysis acuminata* are common later in the summer (Kononen et al. 2003, Hällfors et al. 2011). The summer community in the southern parts of the Baltic Sea is dominated by diatoms (*Skeletonema costatum*, *Thalassiosira baltica*, *Actinocyclus octonarius*), dinoflagellates (*Dinophysis acuminata*, *D. baltica*), cryptophytes (*Hemiselmis virescens*, *Teleaulax acuta*, *Plagioselmis prolonga*), other small flagellates (e.g. *Pyramimonas* spp.) and colonial cyanobacteria (*Merismopedia* spp., *Snowella* spp.) in addition to the filamentous cyanobacteria mentioned above (Maestrini et al. 1999, Yurkovskis et al. 1999, Jóźwiak et al. 2008).

Fall is the transition season between the summer and winter, and lasts from September until the end of November in the Northern Hemisphere. Fall is characterized by lower air temperatures and decreasing day length. In the Baltic Sea, strong winds break up the cyanobacteria surface accumulations during the fall, and the cooling temperature prevents new accumulations from developing (Wasmund 1997). However, during the fall nutrients upwell to the euphotic layer during fall turnover, which enhances phytoplankton production before the winter and can result in a late summer/early autumn bloom. Common species in the Baltic Sea during the fall are the diatoms *Coscinodiscus granii*, *Actinocyclus octonarius*, the dinoflagellate *Dinophysis acuminata* and small colonial cyanobacteria (*Woronichinia* spp., *Snowella* spp., *Aphanothece* spp., *Cyanodictyon* spp.) (Yurkovskis et al. 1999, Wasmund et al. 2001, Hällfors et al. 2011). In late autumn in October diatoms and cyanobacteria may still be common, but in the end of October the microalgal biomass decreases due to the decrease in solar irradiance (Wasmund 1997).

The winter season is the coldest and darkest season in the sub-polar areas, and lasts from December until the end of February. Winter in the northern Baltic Sea is also characterized by sea-ice formation and snow accumulation. Winter is generally described as a dormant phase (Makarevich et al. 2015), but requires further investigation, as only a few frequently sampled data sets exist from the winter season, and these are from the Arctic (Bursa 1961, Levinsen et al. 2000). Data sets exist from the Baltic Sea describing the phytoplankton succession for short time periods during the winter season (Andersson et al. 1994, 1996, Haecky et al. 1998, Rintala et al. 2006, Piiparinen et al. 2010), but frequently collected data sets describing algal succession throughout the winter are still missing. Samples collected during the winter in the Baltic Sea have shown low phytoplankton biomass in the

water column (Andersson et al. 1994, 1996, Haecky et al. 1998, Meiners et al. 2002), indicating phytoplankton dormancy during winter. The winter phytoplankton community includes flagellates from various classes (Meiners et al. 2002). The best available and most frequently sampled winter data sets are from a eutrophic estuary in the southern Baltic Sea, where the winter community is dominated by diatoms, green algae and filamentous cyanobacteria (Schumann et al. 2005).

Sea ice is a suitable environment for a diverse community including viruses, bacteria, microalgae and small animals such as crustaceans (Thomas and Dieckmann 2010), and ice community succession in oceanic sea ice is divided into three successional phases (Leu et al. 2015). In addition to the variability of the biotic characteristics of the sea ice, abiotic parameters are also very variable both regionally and annually. The Baltic Sea ice eukaryotic community resembles the oceanic sea-ice community, but due to the smaller brine volume compared to oceanic sea ice (Meiners et al. 2002) no small animals, such as crustaceans, occur in the Baltic Sea ice. Global debate of whether the sea-ice community is identical to the water column community is still ongoing. Tuschling et al. (2000) and Majaneva et al. (2012) have shown that sea-ice communities differ from those observed in the water column in the newly formed sea ice, whereas Różańska et al. (2008) showed that nearly all species were observed both in the newly formed sea ice and in the water column, while only a few species were found exclusively in the water column. These differing results may partly be explained by the different methods used in species identification, i.e. DNA (Majaneva et al. 2012) and microscopy (Tushling et al. 2000, Różańska et al. 2008). In addition, studies in the Arctic and Antarctic have shown that several diatom species are found in both the ice and on the sediment (Wulff et al. 2008a,b, Al-Handal et al. 2016), and sea-ice diatoms are suggested to originate from benthic diatoms. In the Baltic Sea ice biomass and chlorophyll *a* (chl *a*) concentration increase from the beginning of the ice-covered season towards the end of the season (Haecky et al. 1998, Kaartokallio 2004), and the spring ice community is dominated by diatoms and dinoflagellates (Haecky et al. 1998, Haecky and Andersson 1999, Meiners et al. 2002, Piiparinen et al. 2010, Rintala et al. 2010). Baltic Sea ice is estimated to contribute approximately 10 % of the primary production during the ice-covered season (Haecky and Andersson 1999). However, the Baltic Sea winter sea-ice community has been left largely unexplored and less is known about its succession, and thus more studies are needed for comprehensive understanding of sea-ice algal succession.

1.2. Characteristics and effects of solar radiation on microalgal communities

Most of the solar radiation reaching the Earth's surface is in the range from 100 nm to 1 mm. This wavelength area is divided into three ranges: the ultraviolet range from 100 nm to 400 nm, which is further divided into UVC (100–280 nm), UVB (280–315 nm) and UVA (315–400 nm), visible light from 380 nm to 780 nm and infrared from 700 nm to 1 mm. The most important radiation for photosynthesis is from 400 nm to 700 nm (PAR), which is very close to the range of visible light (380–780 nm). Plants and algae capture PAR with different pigments that are specified to certain wavelengths, but chl *a*, which absorbs light most effectively

at the wavelengths between 440 nm and 670 nm, is the most essential for photosynthesis (Kirk 2011).

Solar altitude, which depends on the time of day and time of year, affects the amount of solar irradiance that penetrates the sea surface. As the solar altitude decreases, the downward irradiance in the water column attenuates more quickly. In an aquatic medium the downward irradiance diminishes with depth due to absorption and scattering. In non-productive waters the water itself absorbs most of the light, whereas the blue area of the spectrum is absorbed by algae in areas with high phytoplankton primary production (Kirk 2011). The crystal structure of the sea ice causes the downward irradiance to react differently compared to the water column. The structure of the ice affects the scattering coefficients and therefore also light attenuation. The granular layer has higher scattering compared to the columnar ice (Uusikivi et al. 2010). In addition, the amount of snow on the ice alters the albedo and the amount of penetrating light (Pirazzini et al. 2006). Ice cover in the Baltic Sea is thinner compared to the Arctic, and PAR transmittances are therefore typically two to four times lower in the Arctic than in the Baltic Sea (Uusikivi et al. 2010).

High energetic ultraviolet radiation is harmful for microalgal cells in several different ways. Ultraviolet radiation damages DNA, reduces microalgal enzyme and protein production, decreases the velocity of cell movement and changes cellular stoichiometry by decreasing the uptake and inhibiting the utilization of inorganic nutrients (Häder and Häder 1991; Döhler 1992; Arts and Rai 1997, Karsten et al. 2009). Mobile microalgae protect themselves against UVR by moving away from it (vertical migration) (Wulff et al. 2008b). However, this is restricted in the sea ice, as the microalgae are trapped in the brine channels. Microalgae also protect themselves against UVR by producing "sunscreens", including photoprotective pigments, such as carotenoids, and MAAs, which are small secondary metabolites (e.g. Helbling et al. 1996; Ryan et al. 2002, Bonilla et al. 2009). However, MAAs are also produced under higher PAR (Karsten et al. 1998, Carreto et al. 2002). More than 20 MAAs, with maximum absorption ranging from 309 nm to 362 nm, have been well characterized, and due to the increasing number of studies and development of methods, previously unknown or only partially characterized MAAs are being detected and described in increasing numbers (Carreto and Carignan 2011). Although MAAs provide protection to organisms against UVR (Karenz et al. 1991b), their concentration per chl *a* and their MAA pool content vary greatly between different microalgae species, but also within the species when measured in individual cultures (Jeffrey et al. 1999).

Sea-ice studies in the Arctic and Antarctic have usually focused on the bottom layer of the sea ice where the highest chl *a* concentrations and microalgal biomass have been observed (McMinn et al. 1999, 2003, Juhl and Krembs 2010, Petrou et al. 2011, Ryan et al. 2012, Alou-Font et al. 2013, Lund-Hansen et al. 2014). Bottom ice is therefore usually considered the most important part of the ice in respect to primary production (PP). Less is known about the importance of the upper parts of the ice column to PP. The environment in the bottom layer of the sea ice is more stable due to both physical and chemical properties, i.e. bottom ice temperature is closer to the water temperature compared to the upper layers, which are more affected by air temperature (Perovich and Gow 1996). In addition, the bottom ice

community is sustained by a nutrient supply from the under-ice water (UIW) (Cota et al. 1987). However, heterotrophic bacteria also play an important role in the nutrient cycle in the sea ice (e.g. Riedel et al. 2007). In addition, the light environment in the bottom ice is quite different compared to the upper layers of sea ice, due to the structure of the ice and the different behaviour of the light in the granular and columnar ice (see next paragraph). The effect of UVA on sea ice algae in the Baltic Sea has been studied throughout the ice column (Piiparinen and Kuosa 2011). However, UVA is the part of the spectrum that penetrates through the atmosphere and ozone layer, whereas UVB is mostly absorbed by the ozone layer, and thus sea ice algae are exposed to different proportions of UVA and UVB radiation. Thus the effect of both UVA and UVB on sea ice algae in the different layers requires further investigation.

1.3. The Baltic Sea and sea ice

The Baltic Sea is one of the largest brackish water bodies (393 000 km^2) located in the temperate and boreal zones in Northern Europe between latitudes 54°N and 66°N. It is completely located on one continental plate, which is why the Baltic Sea is shallow. The mean depth is only 54 m and the water volume is circa 21 000 km^3 (Leppäranta and Myrberg 2009).

The Baltic Sea is a semi-enclosed water body, and its only connection to the North Sea is through the narrow and shallow Danish Straits in the southeastern part of the Baltic Sea. The narrow straights together with the four times larger drainage basin (1 633 000 km^2) and fresh water run-off from > 200 rivers results in a latitudinal salinity gradient of 20 close to the Danish Straits to 1 in the northern part of the Bothnian Bay. In addition to the horizontal salinity gradient, two haloclines are usually present. One halocline is closer to the surface together with the thermocline (15 m depth) while the other deep-water layer is more permanent (60–80 m depth), which is related to salt water pulses that enter the Baltic Sea through the Danish straits at irregular intervals and depending on weather patterns (Voipio 1981, Leppäranta and Myrberg 2009). The strong salinity gradient also leads to low species diversity, and species composition is a mixture of marine and fresh water species (Ojaveer et al. 2010). The phytoplankton community structure differs spatially both in the south-north and coast-open water direction following the salinity gradient (Gasiūnaitė et al. 2005), in addition to the temporal annual algal succession, which follows climatic conditions and differs between the south and north (Niemi 1975).

Annual freezing is one of the special characteristics of the Baltic Sea (Leppäranta and Myrberg 2009). The sea ice has a semi-porous structure, which forms when the salinity of the parent water is more than 0.6 (Palosuo 1961), and therefore the Baltic Sea ice also resembles the ice found in the Arctic and the Antarctic. Sea ice forms from pure water, and the salts in sea water are concentrated, forming a condensed salty liquid called brine, which is trapped between the ice crystals, and ultimately channels and pockets filled with brine are formed (Petrich and Eicken 2010). However, brine volume is salinity dependent, and consequently brine volume and salinity are lower due to the lower salinity of the Baltic Sea compared to the oceans (Meiners et al. 2002). Brine salinity in the brine pockets in Baltic Sea ice can be similar to the salinity of the ocean water column (Meiners et al. 2002). Albeit

the insulating impact of snow cover, changes in ice temperature are most pronounced in the ice surface because the air temperature is more variable than the water temperature (Webster et al. 2014).

In the Baltic Sea and elsewhere ice cover formation differs between coastal and open areas. The ice formed near land and attached to the land is called fast ice. Baltic Sea fast ice typically has a two-layer structure with a thin granular layer on top and a thicker columnar layer below (Kawamura et al. 2001, Granskog et al. 2003). In open areas, where freezing occurs with a wave motion, ice flows scavenge against each other forming round ice floes (pancakes) that eventually freeze together to form an ice sheet (Weeks and Ackley 1982). Freezing of the Baltic Sea begins in the north. The Bothnian Bay freezes over on average in mid-January, and the Bothnian Sea, the Gulf of Finland and the Gulf of Riga freeze one month later (Leppäranta and Myrberg 2009). Ice melting is reversed, starting from the southern parts and continuing towards the northern Bothnian Bay where the ice breaks up during May/June. The extent of the sea-ice cover is at its largest from mid-February to mid-May, when it covers an average 45 % of the Baltic Sea area, but ranges from 12.5 % to 100 % during various years (Leppäranta and Myrberg 2009). However, during the period 1927–2012 the maximum sea-ice cover extent and duration of the ice-covered season has significantly decreased, by nearly 30 days (from 84 to 54 days in the nearshore zone and from 70 to 40 days in the open sea zone) (Merkouriadi and Leppäranta 2014).

2. OUTLINE OF THE THESIS

This thesis is divided into two parts: i) the natural succession of phytoplankton and sea ice algae during the cold-water season and ii) the effect of UVR and enhanced PAR on the fast-ice microalgal communities. Paper I is focused on the microalgal succession in the water column throughout the cold-water season and in the sea ice during the ice-covered period. The main focus in the two following papers (II and III) is to study the effect of enhanced PAR and UVR on sea ice algae in the various ice layers. In paper II the focus is on the effects on sea-ice algal biomass, community composition and photosynthetic activity. Paper III studies the concentration and composition of ultraviolet radiation-absorbing MAAs used for UVR protection by the sea ice algae.

In this thesis the cold-water season refers to the period from late fall until the end of the spring bloom, and winter is referred to as the ice-covered season. During winter, the sea ice is a habitat with abundant microalgal community and biomass increases in the sea ice, whereas the biomass in the water column remains low throughout the winter season. Four major questions are addressed in the first study (I): 1) Is the winter period a dormant phase as previously considered? 2) Is the entire water column microalgal community incorporated into the sea ice during freezing or is the ice community formed by only certain species? 3) Does the young sea-ice algal community determine the ice community for the entire ice-covered season? 4) Do sea ice algae contribute to the phytoplankton community after ice break-up?

Ultraviolet radiation has harmful effects on organisms in terrestrial and aquatic environments. Sea ice algae in the sea ice are especially sensitive when exposed to

UVR, as they are restricted or incapable of moving away from the enhanced solar irradiance. Previous studies have focused on the effects of UVR on sea ice algae in the bottom ice, where the light environment is different compared to the upper layers, due to the thickness of the ice and snow cover on the ice. The aim of these studies (II, III) is to add to our knowledge of the effects of UVR on sea-ice algal biomass, community composition and photosynthetic activity, and to describe the components of UVR protection of the microalgae, especially in the top 10-cm ice layer.

In this study, weekly sampling of natural microalgal communities combined with field experiments provides a better understanding of the natural seasonal succession of microalgae communities during the cold-water season both in the water column and sea ice, and also shows the influence of increased solar irradiance on sea ice algae in the various ice layers.

3. STUDY SITE

This study was carried out on the northwest coast of the Gulf of Finland, the Baltic Sea in northern Europe. In the succession study (I) two different locations were selected as study sites. Krogarviken, site A (59° 50.650′ N, 23° 15.100′ E) is a semi-enclosed shallow bay with average water depth of only 3 m, but with high sea-ice probability and unlikely events of any sea-ice breakup events during the ice-covered season. The site B, Storfjärden (59° 51.250’ N, 23° 15.815’ E), is approximately 30 m deep and more exposed to heavy winds, which can easily cause sudden sea-ice breakups. The UVR experiment (II, III) was performed at site A.

4. MATERIALS AND METHODS

4.1. Sampling and experimental setup

4.1.1 Succession study (I)

The sampling was carried out weekly from October 8th 2012 to May 20th 2013, with the exceptions of December 24th and 31st and during rasputitsa on April 22nd. In addition, due to poor sea-ice conditions, samples could not be collected from the site B in January 7th, or February 11th and 18th.

Three replicate water samples were taken from each site (A: 0–3 m; B: 0–15 m) using a 3-m and 15-m long hose samplers (6 cm internal diameter). The cutoff of 15 m at site B was chosen as a typical depth scale for the euphotic zone (Luhtala and Tolvanen 2013). The water samples were collected into 2-L transparent plastic bottles. After the sea ice had formed, three snow thicknesses on the ice were measured from three random spots with 1-cm precision, followed by ice sampling using a motorized CRREL-type ice-coring auger (9 cm internal diameter; Kovacs Enterprises, Roseburg, OR, USA). Sea-ice temperatures were measured at 5-cm intervals using a Testo 110 thermometer and sea-ice thicknesses were measured from the obtained ice cores before they were placed in plastic tubing (Mercamer Oy, Vantaa, Finland). Three replicate ice samples were taken from each site. For one replicate ice sample, 2–5 ice cores, depending on the ice thickness, were drilled and pooled to ensure enough melted sea ice comparable to the 2-L water samples. In addition, three replicate under-ice water (UIW) samples were collected from the drill hole simply by submerging the 2 L plastic sample bottle under the water's surface. All of the obtained water and sea-ice samples were kept in the dark during transportation to the field station, where the sea-ice samples were crushed and melted without allowing the temperature of the sample to rise above +4◦C, as explained in Rintala et al. (2014) (I).

4.1.2 UVR experiment (II, III)

The UVR experiment was carried out as a 3-week *in situ* experiment at site A. The experimental field was established on February 28th 2011 when there was from 33 to 38 cm of thick ice covered uniformly by 4–6 cm of snow. The experimental setup consisted of nine 1.44 m^2 squares, about 1 m apart from each other and divided into three treatments (three replicates each) arranged in a 3 × 3 Latin square design to account for potential horizontal patchiness (Fig. 1). Three of the squares had natural snow cover throughout the experiment (untreated, UNT). Snow was removed from the remaining six squares, and three of them were exposed to the natural solar spectrum (PAR+UVR treatment), and the three remaining squares (PAR treatment) were covered with UV filter film (No. 311413; Roscolab Ltd, London, England), which blocked the radiation below 390 nm but allowed about 82 % transmission of wavelengths >390 nm (Piiparinen & Kuosa 2011). Since the wind constantly carried some loose snow over the experimental field, the PAR and PAR+UVR treatments were cleared of snow and frost every day throughout the experiment. After the sampling on day 14 (Mar 14th), the UV filter film was removed from the PAR treatment re-exposing the ice to the full solar spectrum (PAR (+ UVR)).

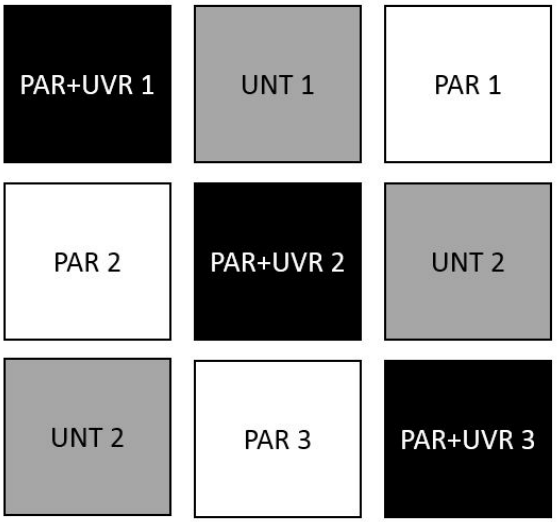

Fig. 1. Latin square experimental design for the UVR-experiment (papers II and II), including three replicate squares of each three treatments. Three squares were covered with natural snow cover (UNT). The remaining six squares were without snow cover, and three of them were exposed to the natural solar spectrum (PAR + UVR), and the three remaining squares (PAR) were covered with UV filter film.

Ice samples were collected from all treatments every 7 days (Feb 28th, Mar 7th, Mar 14th and Mar 21st), except for the last sampling (21 d) when ice samples were collected only from UNT and PAR(+UVR) treatments in order to study the effect of re-exposing the ice to the full solar spectrum during the third week of the UVR experiment. The ice samples were collected using the same ice coring auger as in the succession study (I). To ensure enough ice for all analyses and to reduce the effect of patchiness, five ice cores from each square were taken (0 d, 7 d, and 14 d). The core holes were sealed with frozen fresh ice cylinders to prevent damage to the sampling field, e.g. from lateral brine drainage via the drill holes. On day 21 only one ice core per replicate sample was collected because the volume of melted ice from one ice core was enough for the analyses. The thickness of each ice core was measured to 1-cm precision, and the ice cores were cut into four vertical pieces. The impact of UVR was assumed to be greatest in the surface layers of the ice, and thus the topmost 10-cm layer was sliced into two 5-cm layers. The bottommost 10-cm layer represented the bottom ice community and the remaining intermediate parts of the cores were from 13 to 20 cm thick. After slicing the ice cores, the five replicate ice pieces per layer in each square were pooled in one sample (except 21 d when only one ice core) and placed in plastic containers or in plastic tubing (Mercamer Oy, Vantaa, Finland). The ice samples were kept in the dark during transportation to the field station, where they were treated in the same way as in the succession study (II, III).

The number of true replicates (n=3) (I-III) and the pooling of the samples (n=1) on day 21 in the UVR experiment (III) for the measurements of photosynthesis-irradiance, mycosporine-like amino acids, nutrients and operational taxonomic units were chosen due to the available facilities. Consequently, the low number of the true replicates should be considered for possible error sources when interpreting the results.

4.2. Measurements

4.2.1 Physico-chemical variables

Temperature and salinity of the water column were measured using a Falmouth Scientific NXIC CTD equipped with WET Labs ECO fluorometer sensor (I). The bulk salinities of the melted sea-ice samples were

measured with a YSI 63 meter (Yellow Springs Instruments Inc., Yellow Springs, OH, USA) (I-III). In the UVR experiment the ice surface temperature in all treatments was measured with three aluminium foil-covered temperature loggers (Hobo Pro v2; Onset Computer Corp., Bourne, MA, USA) (one logger per treatment) at 1-h intervals throughout the first two weeks of the experiment (Feb 28th – Mar 14th, 2011). The loggers were placed between the ice surface and the snow (UNT), on top of the ice (PAR+UVR) and between the ice surface and the film (PAR).

For nutrient analysis an equal amount of water from each replicates was pooled into one sample. Both inorganic (NH_4-N, NO_2+NO_3-N, PO_4-P, and SiO_4-Si) and total nutrient (tot-N and tot-P) concentrations were determined using a Hitachi U-110 Spectrophotometer (Hitachi High-Technologies Corp., Tokyo, Japan) with standard protocols for seawater analysis (Koroleff 1976). Ice nutrient concentrations were normalized to the mean bulk salinities of melted sea ice to correct for salinity-related variations in the nutrient concentrations (I-III).

4.2.2 Irradiance measurements

The incoming spectral irradiance during the first 14 day of the UVR experiment (II, III) was measured from 280 to 800 nm in 1-nm steps at 1-h intervals with a Macam SR991 spectroradiometer [Macam Photometrics Ltd (now Irradian Ltd), Tranent, West Lothian, UK] and air temperature at 0.5-h intervals with a GroWeather station (Davis Instruments Corp., Hayward, CA, USA). Both instruments were placed on the roof of Tvärminne Zoological Station about 200 m from the UVR-experiment field.

4.2.3 Chlorophyll a measurements, microalgal identification, cell enumeration and biomass

For measuring chl *a* concentration, two 100-mL subsamples were taken from every water and ice sample. They were filtered onto GF/F filters (Whatman, Sigma-Aldrich Co. LLC, St. Louis, MO, USA), soaked in 96 % v/v ethanol and kept in darkness overnight to extract chl *a*. The chl *a* concentration was calculated from the chl *a* fluorescence measured with a Cary Eclipse spectrofluorometer (Varian Inc. (Agilent Technologies), Santa Clara, CA, USA) calibrated with pure chl *a* (HELCOM 1988). In the succession study (I), the chl *a* concentrations for the ice were converted to mg chl *a* m^{-3} of sea ice by multiplying the chl *a* concentration of the melt water by a standard sea ice to seawater density ratio (917 kg m^{-3} / 1020 kg m^{-3} = 0.9). In addition, on day 21 in the UVR experiment (III), the three replicate samples (treatments UNT and PAR(+UVR)) were pooled, because the chl *a* results were reported in proportion to MAA concentrations, which were measured from the pooled samples as well (see section 4.2.5).

For microalgal identification, cell enumeration and biomass estimation, 200-mL subsamples were collected into a brown glass bottle from every sample, preserved with acid Lugol's solution and stored refrigerated in darkness until microscopic enumeration. Depending on the sample's microalgae density, a volume of 50 mL or 10 mL was settled for 24 h, according to Ütermöhl (1958), and examined with a Leica DM IL, Leica DMIRB, Leitz DM IL, Olympus CK30 or Olympus CKX41 inverted light microscope equipped with 10x oculars and 10x or 40x objectives (Leica Microsystems, Wetzlar, Germany; Olympus Corporation, Hamburg, Germany). Large

cells and colonies were counted with 100x magnification over an area that covered one half of the cuvette, and the abundance of single-celled and small taxa was counted from 50 random fields with 400x magnification (HELCOM 2008). The species with morphological characteristics visible in an inverted microscope, e.g. with easily recognizable colony structure and cell shape, were identified to species level whereas microscopically unidentifiable species were left to a general level. Species easily identified incorrectly (*Gymnodinium corollarium*, *Biecheleria baltica* and *Scrippsiella hangoei*) due to similar gross morphology were identified as the *Scrippsiella* complex in the acid Lugol's fixed samples. The cell numbers were converted into carbon biomass (mg C m^{-3}) using species-specific biovolumes and carbon contents according to Olenina et al. (2006) and Menden-Deuer & Lessard (2000). In the succession study (I) the microalgal biomass in the ice was converted to mg C m^{-3} of sea ice in a similar manner as the chl *a* used for ice (see above). On day 21 in the UVR experiment (III) the three replicates (treatments UNT and PAR(+UVR)) were pooled.

4.2.4 Photosynthesis-irradiance measurements

Photosynthetic activity was examined as a photosynthesis-irradiance response. The samples were pooled from each replicate water and ice sample (I) and ice layer from the three replicate squares (0 d, 7 d, 14 d) (II). The method of Steemann Nielsen (1952) with modifications by Niemi et al. (1983) was used for calculating the carbon assimilation. Sample volumes of 3 mL with 50 µL $NaH^{14}CO_3$ addition (final concentration 0.33 µCi mL^{-1}) were incubated for 2 h under 16 different light intensities between 6 and 4087 µmol photons m^{-2} s^{-1} with two dark controls in incubators cooled with cold water circulation. The highest light intensities in the incubators were twice the natural irradiance, which at the surface of the Baltic Sea can be as much as 2000 µmol photons m^{-2} s^{-1} in summer (Müller 2004). The incubation was stopped by adding 100 µL of 37 % formaldehyde to the samples. The unincorporated $NaH^{14}CO_3$ was removed from the samples during the following 48 h by addition of 100 µL of 1 N HCl. Insta-Gel Plus (PerkinElmer, Waltham, MA, USA) scintillation cocktail was added, and the incorporated radioactivity was measured with a Wallac Win Spectral 1414 scintillation counter (Wallac PerkinElmer, Turku, Finland). The total inorganic carbon was measured, using a Uras 3E carbon analyser (Electro-Dynamo AB, Helsingborg, Sweden), as explained by Salonen (1981). The carbon uptake rates were normalized to chl *a* (mg C [mg chl *a*]$^{-1}$ h^{-1}) for the UVR experiment (II). In addition, the photosynthetic efficiency (α^b), maximum photosynthetic capacity (P^bm), photoinhibition (β) and the light saturation index (E_k) were determined from photosynthesis-irradiance response curves, according to Platt et al. (1980). The superscript 'b' for α^b and P^bm denotes the normalization to chl *a*.

4.2.5 Mycosporine-like amino acids (MAAs) (III)

For MAA analysis (III), the three replicate samples were pooled by treatment, and 300 mL of each sample was filtered onto a GF/F filter (Whatman) and the filters were stored at −20 °C for 8 months prior to extraction according to a protocol described by Tartarotti & Sommaruga (2002). The

filters were sonicated (Sonopuls HD 2070; Bandelin, power 70 W, frequency 20 kHz, 40 % amplitude) in 800 µL of 25 % MeOH (v/v) for 30 s on ice, incubated at 45 °C for 2 h and placed in −80 °C overnight. After this, the samples were allowed to reach room temperature, the filters were removed with forceps and the extracts were centrifuged (16 000g) for 20 min at +4 °C. MAAs were analyzed by injecting 80 µl of extract into a reversed-phase high-performance liquid chromatography (Dionex) equipped with a 250 mm × 4.6 mm Phenosphere 5 µm C8 column and a guard column (Phenomenex). Samples were run at 0.75 mL min−1 flow rate using 0.1 % acetic acid in 25 % MeOH (v/v) as mobile phase and the absorbance between 200 and 595 nm was measured with a Dionex diode-array detector. The linear response of the diode array detector within the analytical range considered was verified by injecting five different volumes of a purified usujirene/palythene standard (provided by J. I. Carreto). The MAA compounds were identified by comparing their retention times and absorbance characteristics to the purified MAA standards of porphyra-334, shinorine and palythine. The MAA concentrations were calculated from HPLC peak areas at 310, 320, 334 and 360 nm using the published molar extinction coefficients (Takano et al. 1978, 1979, Tsujino 1980). For unknown UVR-absorbing compounds, which had their absorption maxima at 320 nm, 333 nm and ca. 335/360 nm, the extinction coefficients were calculated assuming the same molar composition as the compounds with similar absorption maxima.

4.3. DNA isolation and 18S rRNA gene identification (I)

For the DNA extraction, 500 or 1000 mL of water and melted sea ice was sequentially filtered with 47 mm diameter 180-µm pore-size nylon filters (Merck Millipore, Billerica, MA, USA), 20-µm Polyvinylidene fluoride filters (Durapore©, Millipore) and 0.2-µm mixed cellulose ester membrane filters (Schleicher and Schuell Bioscience GmbH, Dassel, Germany). The filters were stored in a -80°C freezer until further processing. Total DNA was extracted from the 0.2-µm filters, using a PowerSoil® DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA, USA).

PCR amplification was carried out in two stages following Koskinen et al. (2011). In brief, the V4 region of the 18S rRNA gene was amplified, using Phusion polymerase (Finnzymes, Espoo, Finland) and forward primer E572F (Comeau et al. 2011) with truncated Illumina 5' overhang 5'-ATCTA-CACTCTTTCCCTACACGACGCTCTTC-CGATCT-3' and reverse primer 897R (Hugerth et al. 2014) with 5' overhang 5'-GT-GACTGGAGTTCAGACGTGTGCTCTTC-CGATCT-3'. Cycling conditions consisted of an initial denaturation at 98 °C for 30 s, 20 cycles of 98 °C for 10 s, 65 °C for 30 s, and 72 °C for 10 s, and a final extension for 5 min. The amplification was completed in three replicates during the second stage, using a full-length Illumina P5 adapter and Indexed P7 adapters. The replicates were pooled between and after the amplifications. The PCR products were purified using AMPure XP beads (Beckman Coulter Inc., Brea, CA, USA) and quantified with Qubit (Invitrogen, CA, USA). The amplicons were paired-end sequenced on an Illumina MiSeq instrument using a v3 600-cycle kit (Illumina, CA, USA)

at the Institute of Biotechnology (Helsinki, Finland).

The resulting reads were processed, using usearch v8.1.1831_win32 (Edgar 2013). In brief, the paired-end reads were merged using -fastq_mergepairs and quality filtered using a -fastq_filter with a minimum read length of 200 bases and a 1.0 maximum expected error rate. The primer sequences were removed and the reads were dereplicated, using -derep_fulllength. Singletons were removed and operational taxonomic units (OTU) were clustered at 97 % similarity level, using -cluster_otus. The above steps were performed for each sample, and the resulting OTU fasta-files were pooled using merge.files in mothur (Kozich et al. 2013). The OTUs were next sorted based on the abundance of reads assigned to them, using usearch command -sortbysize. To remove duplicate OTUs stemming from taxa present in several samples, the merged OTUs were re-clustered at 97 % similarity level, using -cluster_otus -minsize 2. The abundance of each OTU in each sample was resolved, using -usearch_global against the pooled OTU-file. This pipeline was developed based on an analysis of a mock community and seven negative PCR reactions sequenced together with the samples (M Majaneva unpubl. data). The identity of the 97 % OTUs was searched, using classify.seqs in mothur against the PR^2 reference library (Chevenet et al. 2006, 2010, Guillou et al. 2013) and using blastn search in BLAST 2.3.0+ (Zhang et al. 2000) against a nucleotide database at the National Center for Biotechnology Information (NCBI), followed by the lowest common ancestor algorithm in MEGAN6 (minimum bit score 400, top percentage 3.0 and minimum support 1; Huson et al. 2016). Taxonomy was assigned based on an agreement between the two searches. In the absence of a taxonomic assignment, the OTU was treated as unclassified and removed from the further analyses as a putative chimera (372 OTUs).The OTUs assigned as Metazoa, land plants and land fungi were also removed for the downstream analyses (41, 10 and 38 OTUs, respectively), and the number of reads/sample was normalized to 27591. Diversity metrics (ACE, Shannon and inverse Simpson's indices based on abundance of OTUs) were calculated, using the summary. single command in mothur.

4.4. Statistical testing

4.4.1 Succession study (I)

The paired t-test was used to test the significance of differences between the sites for mean water temperature and salinity, snow cover thickness and ice thickness. One-way analysis of variance (ANOVA) was used to test the significance of the differences between the sites and between ice and water for chl *a* and diversity metrics. Levene's test was used to test the homogeneity of variances, using the significance level $p < 0.05$. A parametric ANOVA and Tukey's b test in pairwise comparisons were used when the variances were homogenous, while a non-parametric Kruskal–Wallis test with ranked data and the Mann-Whitney U test were used when the variances were unequal. The correlation between microalgal biomass and chl *a* concentrations was analysed with Spearman's rank-order correlation, using the significance level $p < 0.05$. The procedures were performed in SPSS for Windows (version 23, IBM SPSS Statistics 2015; IBM Corp., Armonk, NY, USA).

To divide assemblages from the operational taxonomic units (OTU) and microscopy analyses into different groups,

the PRIMER v6.1 package of the Plymouth Marine Laboratory and the programme PAST 3.04 (Hammer et al. 2001) were used to perform the multivariate analyses (71 samples). Microalgae and OTUs observed more than once in at least two samples were included in the analyses to ensure sufficient data for ordination, thus reducing the total number of taxa from 76 to 40 (microalgae) and from 1137 to 661 (OTUs). Data on algal biomasses and OTU abundance were log(x+1) transformed to produce a less severe transformation for small values. Groups were defined *a priori* based on substrate and time (water-ice, fall-winter-spring) and hierarchical cluster analysis was conducted on the taxon composition between the samples (Bray-Curtis' similarity index) and tested for significant differences using one-way PERMANOVA with 9999 permutations (Anderson 2001).

4.4.2 UVR experiment (II, III)

A three-way analysis of variance (ANOVA), designed for Latin squares (treatment, row and column as fixed factors), was used to test the significance of the differences between treatments for chl *a*, total biomass and the biomass of most abundant microalgal taxa. Levene's test was used to test the homogeneity of variances, using the significance level $p < 0.05$. A parametric ANOVA and Tukey's b test in pairwise comparisons were used when the variances were homogenous, while a non-parametric Scheirer–Ray–Hare test with ranked data and the Mann-Whitney U test were used when the variances were unequal. The correlation between microalgal biomass and chl *a* concentrations was analysed with Pearson's correlation, using the significance level $p < 0.05$. For testing the differences in the temporal trends of MAA:chl *a* ratios between the three treatments, multiple linear regression models were fitted to MAA:chl *a* ratios normalized to the values on day 0 time, two dummy variables coding the treatments and interaction terms as independent variables. All the procedures were performed in SPSS for Windows (version 22, IBM SPSS Statistics 2013; IBM Corp., Armonk, NY, USA).

5. RESULTS AND DISCUSSION

5.1. Chlorophyll *a* concentration as a proxy for total microalgal biomass

The chl *a* concentration in the water column remained low during the succession study (I) from October 2012 until the beginning of April 2013, and the highest chl *a* concentration was observed at site A just before ice break-up (Fig. 2). Just prior to ice break-up and during the spring bloom the chl *a* concentration in the water column was tenfold compared to the chl *a* concentration during winter. The chl *a* concentration measured from the bulk ice revealed a higher chl *a* concentration compared to the spring bloom, especially at site A (Fig. 2). The temporal variation in chl *a* concentration at site B differed from site A, and the highest chl *a* concentration was observed at the end of February, after ice-cover reformation (Fig. 2).

When the vertical distribution of chl *a* in the spring ice was measured as part of the UVR experiment at site A in 2011, the results revealed an evenly increasing chl *a* concentration from the ice surface to the ice bottom between February 28th and March 14th in the UNT treatment (Fig. 3a,d,g). When the snow cover was removed and the sea ice was exposed to the full solar spectrum (PAR+UVR treatment), an increasing chl *a* concentration from the ice surface to the ice bottom was also observed, but the mean chl *a* concentration in the top 10-cm layer was lower compared to the UNT treatment (Fig. 3e,h). However, when UVR was removed (PAR treatment), the chl *a* concentration was evenly distributed between the ice layers on day 14 (Fig. 3i), suggesting the UVR-induced inhibition of photosynthesis in the PAR+UVR treatment, especially in the top 10-cm layer of the ice.

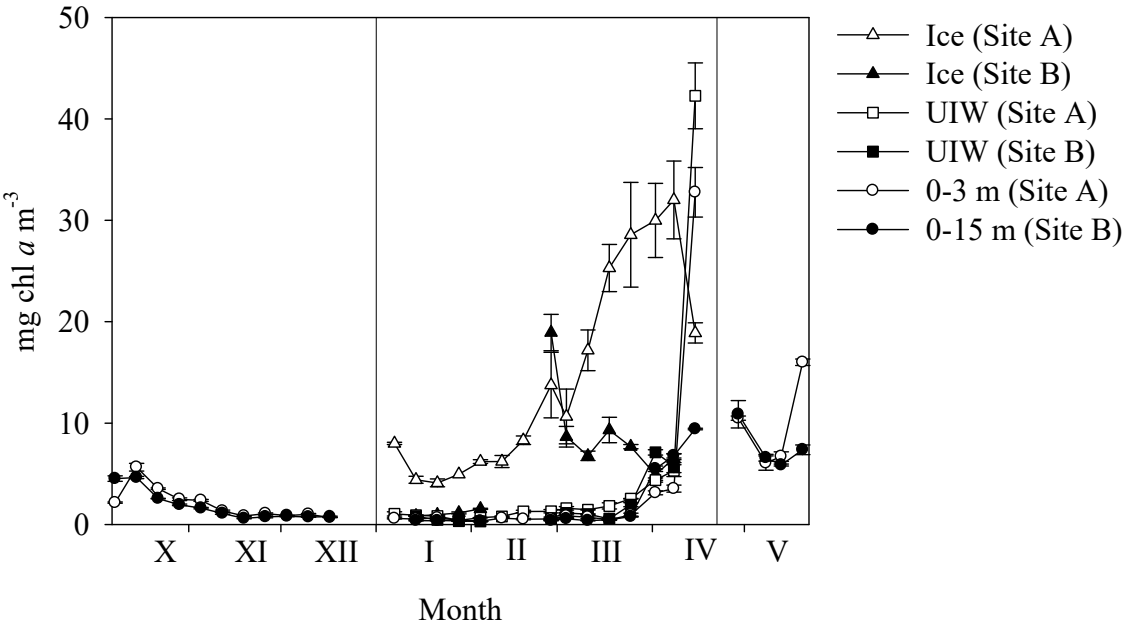

Fig. 2. The chl *a* concentration (mean ± sd, mg chl a m^{-3}) throughout the cold-water season from the beginning of October 2012 until the end of May 2013 in ice, under-ice water (UIW) and the 0–3-m layer at Krogarviken (site A, white) and in ice, UIW and the 0–15-m layer at Storfjärden (site B, black).The timeline from the beginning of January to the end of April represents ice-covered season (I).

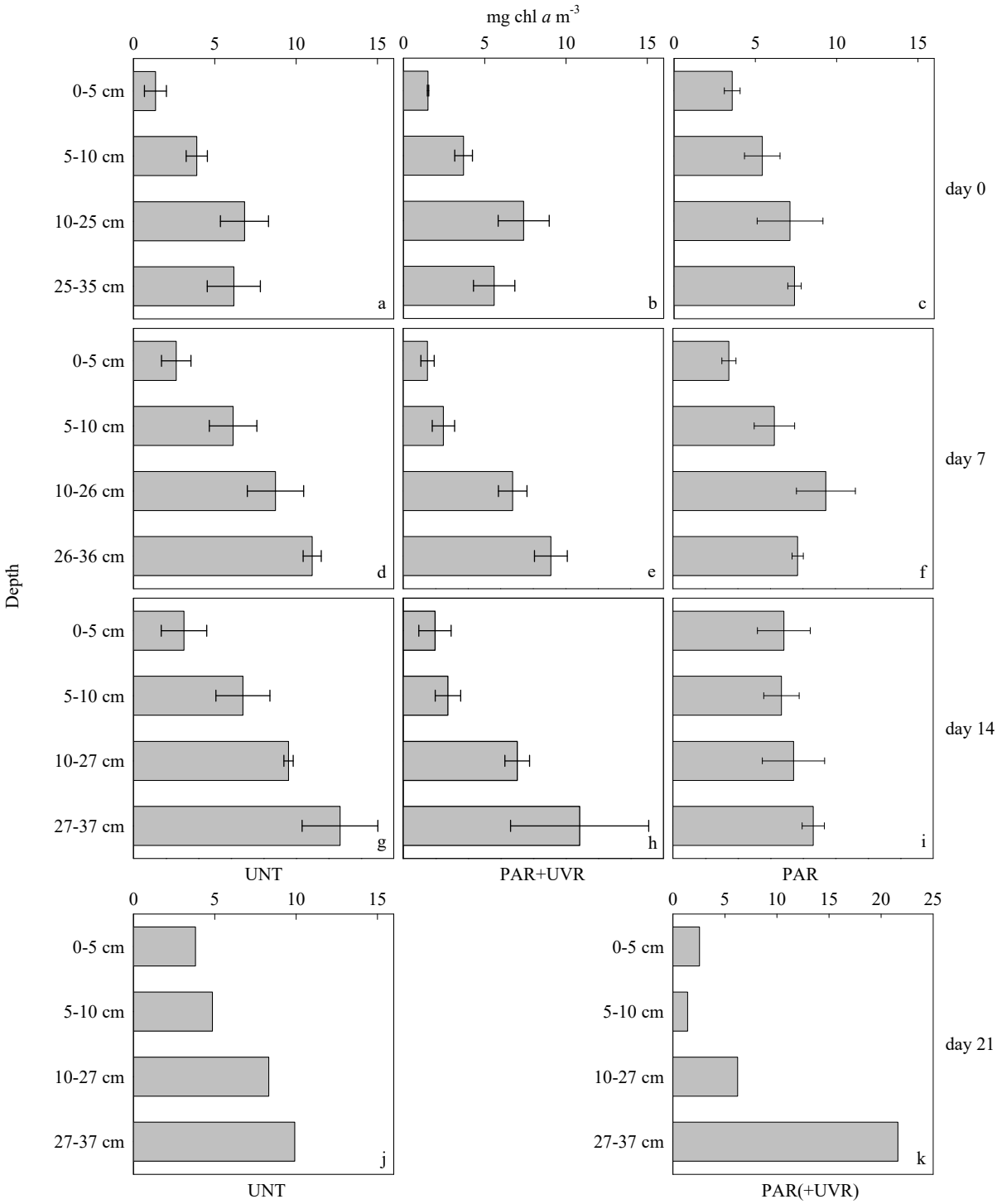

Fig. 3. The chl a concentration (mean ± sd, mg chl *a* m^{-3}) in the treatments with natural snow cover (UNT), exposed to the full solar spectrum (PAR+UVR) and covered with UV filter foil (PAR) in each ice layer at each sampling of the UVR-experiment. Note different scale for x axis in the PAR(+UVR) treatment on day 21 (II, III).

Chlorophyll *a* concentration is frequently used as a proxy for total phytoplankton biomass due to its inexpensive and fast analytical methodology compared to other methods e.g. microscopy and DNA methods. Vörös and Padisak (1991) showed that chl *a* concentration correlates well with biomass estimation. Chlorophyll *a* concentration additionally correlated positively with biomass during the succession study (I) (Spearman's rank-order correlation, $n = 109$, $r^2 = 0.822$, $p < 0.01$) and UVR experiment (II) (Pearson's correlation, $n = 108$, $r^2 = 0.63$, $p < 0.01$). Problems still exist concerning chl *a* concentration used as a proxy for biomass. Firstly, the chl *a* concentration does not give information concerning community composition. Secondly, due to the photoadaptation of the microalgae, the chl *a* concentration may give a false estimation of the total biomass, especially during winter when the amount of light is limited (Falkowski and Owens 1980). This was seen in the UVR experiment, where chl *a* correlated weakly with microalgal biomass. The chl *a*:C -ratio is also different for different species and different situations (Kruskopf and Flynn 2006). All in all, chl *a* concentration as a proxy for biomass gives a useful estimation for the total biomass, but more in-depth studies are needed to gain accurate understanding of species succession. In addition, in areas with sea-ice cover, remotely measuring biomass as chl *a* is difficult, as chl *a* concentration cannot be measured from the sea ice or from the UIW.

5.2. Natural succession of phytoplankton communities during the cold-water season

Sub-polar seas undergo various seasons, including the cold-water season with decreasing air and water temperature, decreasing solar irradiance and possible formation of sea ice. Sea-ice cover limits the amount of light in the water column and thus lowers PP. Samples for microalgal studies during the cold-water season are usually collected infrequently and show low microalgal biomass compared to the phytoplankton biomass during spring and summer (Levasseur et al. 1984, Andersson et al. 1994, 1996, Haecky et al. 1998, Rintala et al. 2006, Mikkelsen et al. 2008, Piiparinen et al. 2010). During fall 2012 the water column (0–3 m at site A and 0–15 m at site B) was characterized by low microalgal biomass (Fig. 4) and the community was dominated by dinoflagellates, cryptophytes and unidentified small (< 20-µm) flagellates (Fig. 5e,f). Analysis of the operational taxonomic units (OTU) revealed that these small flagellates belonged to several different groups i.e. green algae, chrysophytes, cryptophytes and haptophytes (I).

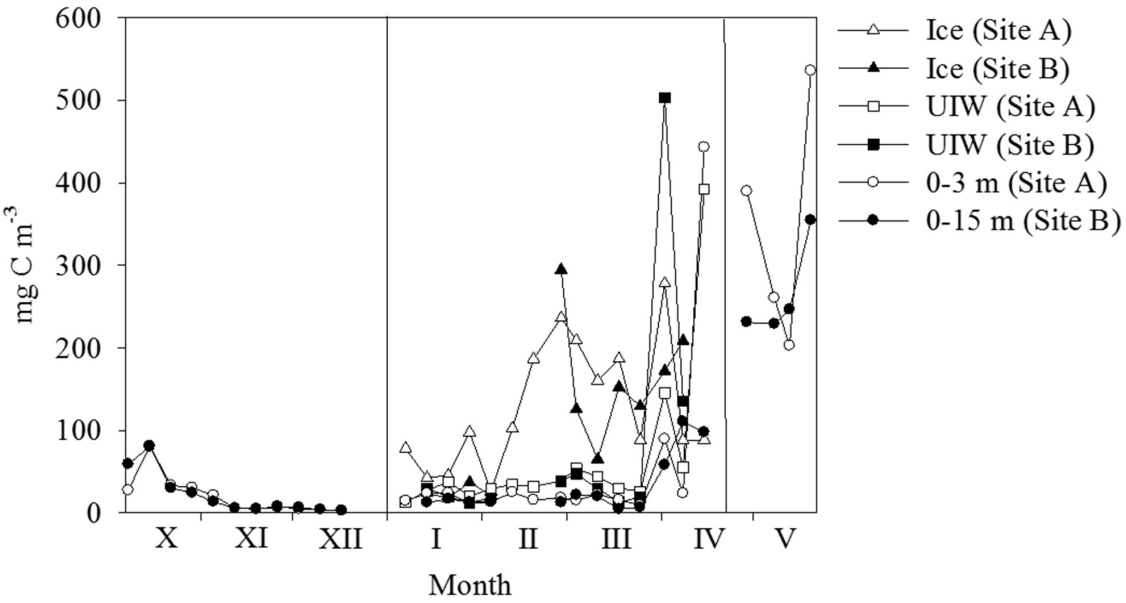

Fig. 4. Total algal biomass (mg C m^{-3}) throughout the cold-water season from the beginning of October until the end of May in ice, under-ice water (UIW) and the 0–3-m layer at site A (white) and in ice, UIW and the 0–15-m layer at site B (black). The timeline from the beginning of January to the end of April represents the ice-covered season (I).

Despite the low microalgal biomass in the water column, the cold-water season could be divided into characteristic groups based on the OTU and microscopy results, suggesting that the wintertime is not a dormant season as previously considered (Krawczyk et al. 2015). The dendrogram produced from Bray-Curtis similarity coefficients of the OTUs (size fraction 0.22–20 μm) showed eight clusters at ~58 % similarity (Fig. 6a). The clusters based on the OTUs followed the a priori groups: the fall (Oct–Dec), the water column during the ice-covered season (Jan–Apr), the UIW (Jan–Apr), the spring bloom (Apr–May) and the ice (Jan–Apr). The microalgal community composition in the sea ice is presented and discussed in section 5.3.1. The groups showed significantly different community compositions (one-way PERMANOVA F = 22.76, sum of squares = 12.04, within group sum of squares = 5.062, $p < 0.001$). Three extra clusters included five transitional samples: two early UIW samples, two early sea-ice samples and one water sample from the winter-spring transition (C14, F14, D13, D14 and F26 in Fig. 6a). In addition, the first three UIW samples (F12, C13, F13 in Fig. 6a) clustered with the winter water samples and the last three winter water samples (A25, E25, E26 in Fig. 6a; sampled under ice) clustered with an early spring water sample. The dendrogram based on the microalgal biomasses (including microalgae > 20 μm) showed similar clustering based on the OTUs (Fig. 6b), and the a priori groups based on microscopy results showed significantly different community compositions, except the fall and winter water (one-way PERMANOVA F = 10.23, sum of squares = 12.26, within group sum of squares = 7.572, $p < 0.001$, Bonferroni corrected). The differences between groups were not as clearly based on microalgal biomass results as they were for the OTU results, but the similarity matrixes, which were used for producing the dendrograms, were related (ρ=0.5, $p < 0.01$) (I).

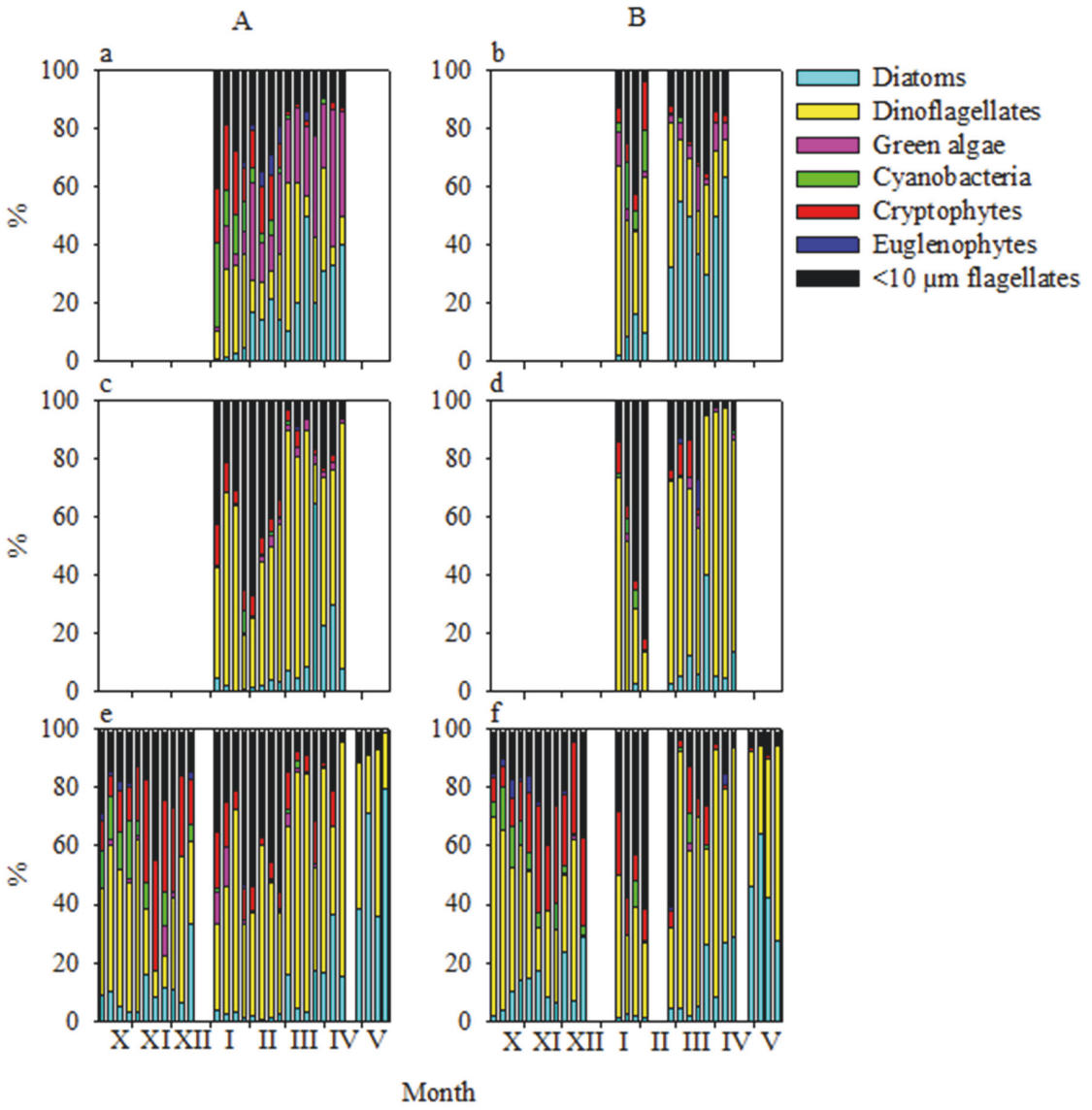

Fig. 5. Seasonal succession of the microalgae assemblage composition (% of the total biomass) from the beginning of October 2012 until the end of May 2013 in ice (a), under-ice water (UIW, c) and the 0–3-m layer (e) at Krogarviken (A) and in ice (b), UIW (d) and the 0–15-m layer at Storfjärden (B) (I).

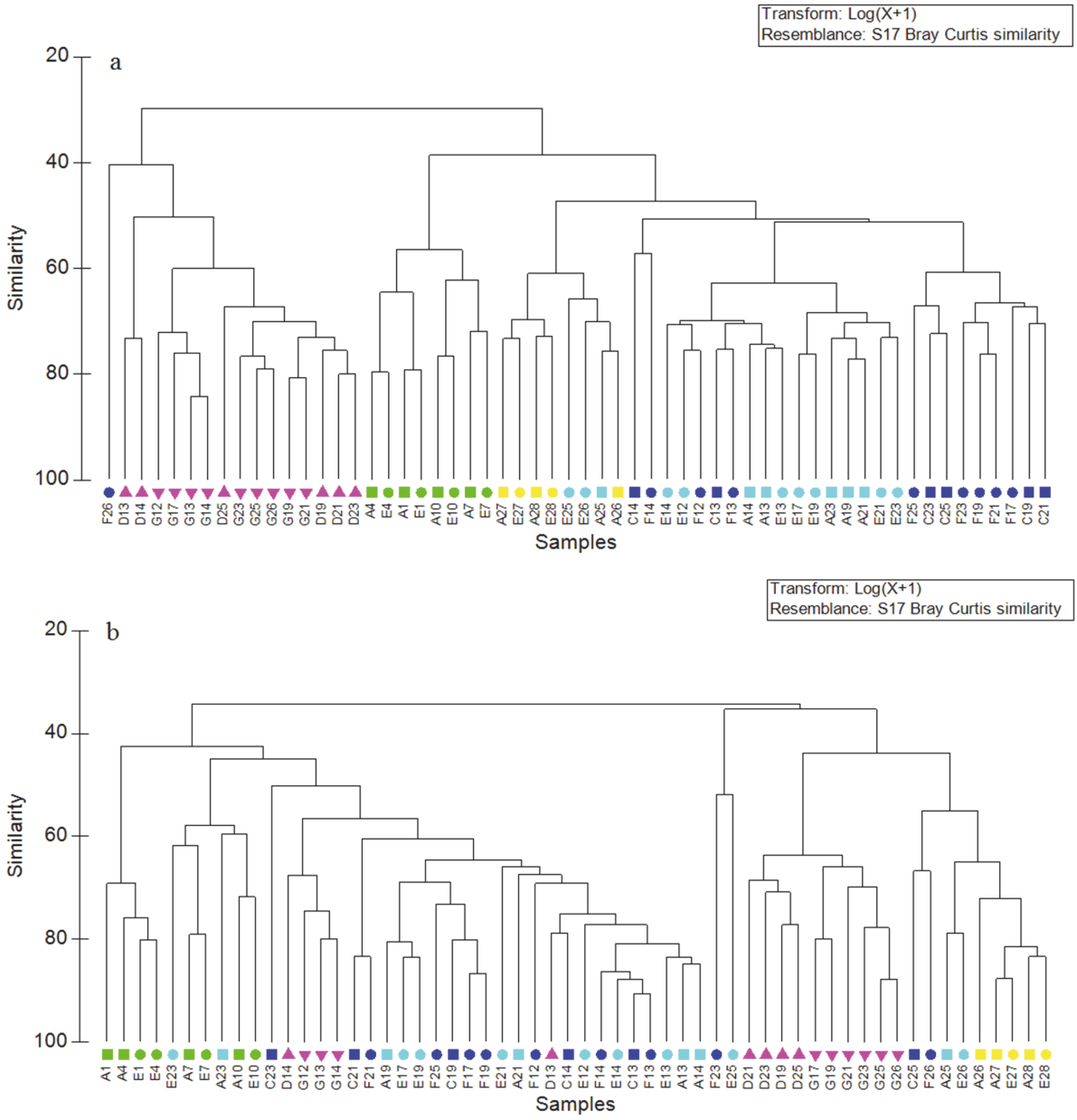

Fig. 6. Dendrograms for percent similarity produced from the Bray-Curtis similarities for OTUs (a) and microscopy results (b). Groups for the fall (green), winter (light blue), ice (pink), UIW (dark blue) and spring (yellow) for Krogarviken (site A, circles and triangles facing down) and Storfjärden (site B, squares and triangles facing up) (I).

The microalgal biomass measured as carbon content in the water column during the ice-covered season in 2013 was lower compared to the fall, but the dominant groups contributing most to total biomass were the same during fall and winter (Fig. 5e,f). Similarly, in previous studies, the water column after ice formation has been characterized by low microalgal biomass and the flagellate-dominated phytoplankton community (Garrison et al. 1993, Meiners et al. 2002, Mikkelsen et al. 2008). The abundance of flagellates during winter is proposed to be related to the flagellates' mixo- and/or heterotrophic ability in addition to photosynthesis (Mikkelsen et al. 2008). However, flagellate dominance during early winter cannot be explained solely with the flagellates' heterotrophic ability, as diatoms also have the capability for heterotrophic growth (Lewin 1953). Decreasing snow cover thickness along with decreasing sea-ice cover thickness result in an increased amount of irradiance in the UIW (Nicolaus et al. 2012). This is the case for example during the spring. Day length increases after the mid-March (after the solar equinox) in the southwest coast of Finland. Despite the ice cover, the biomass in the UIW and water column (0–3 m at site A and 0–15 m at site B) increased and the community changed from flagellate dominated to dinoflagellate and diatom dominated at the beginning of March (Fig. 5c–f). The community in the UIW at both sites was dominated by dinoflagellates, especially species from the *Scrippsiella* complex. Small flagellates, especially *Pyramimonas* sp., were abundant in the UIW of site A. The under-ice blooms are well known under the spring ice, and in the Arctic this period is characterized by extensive diatom blooms in the UIW (Arrigo et al. 2014). These extensive under-ice blooms were linked to massive upwelling events (Spall et al. 2014), thickness of snow and sea-ice cover and the area of melt ponds on the ice (Palmer et al. 2014). Based on the dendrogram results, the community in the UIW was distinctly different from the water column and sea-ice communities (I). This indicates that microalgal community composition under the sea ice is unique and formed only when sea ice is present. In addition to sea ice algae, the phytoplankton under the ice may play an important role in the ice-covered areas where microalgae, both in the sea ice and UIW, provide a food resource for animals at higher trophic levels.

The spring bloom period is a widely studied period in the Baltic Sea (Wasmund et al. 1998, Fleming and Kaitala 2006, Lips et al. 2014). After the severe ice winter 2012–13 the spring bloom was dominated by dinoflagellates and diatoms (Fig. 5a,b), whereas the community after a mild winter is dominated by dinoflagellates (Klais et al. 2011). The dominant dinoflagellates in the spring blooms in the Gulf of Finland are members of the *Scrippsiella* complex and *Peridiniella catenata* (Wasmund et al. 1998, Höglander et al. 2004). Both dinoflagellates were also abundant during the spring bloom of 2013. *Skeletonema* col., *Chaetoceros* spp., *Thalassiosira* spp. and *Pauliella taeniata* are the dominant diatoms during the spring blooms observed in previous studies (Kuosa et al. 1997, Haecky et al. 1998, Höglander et al. 2004). *Pauliella taeniata* was present in spring 2013 right after ice break-up, indicating the seeding effect from the sea ice, but the species did not dominate the diatom community during the spring bloom. The spring bloom diatom community was initially dominated by *Skeletonema* col. and later by *Diatoma tenuis*, neither of which was found to be abundant in the sea ice (I). This indicates

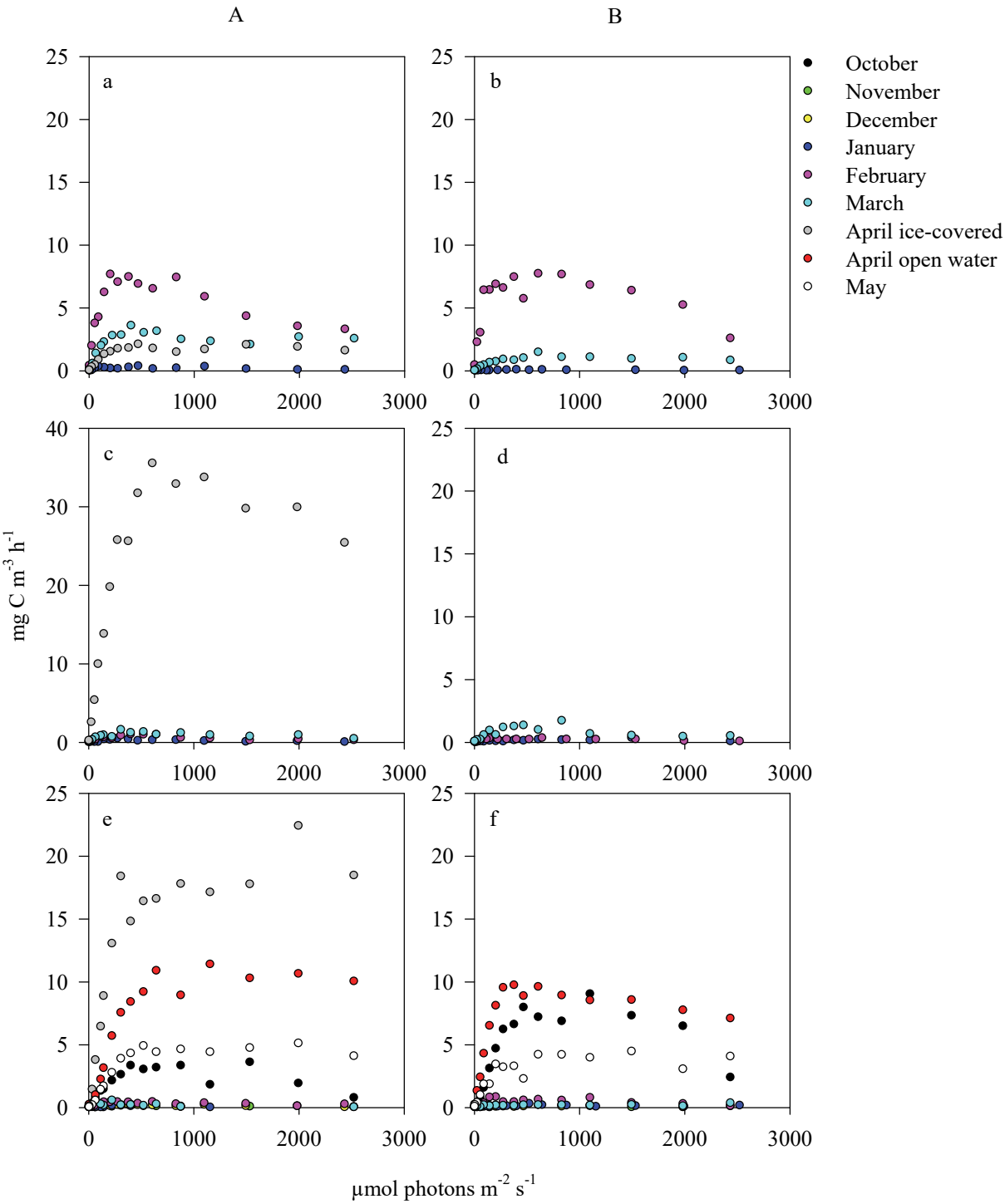

Fig. 7. Photosynthesis-irradiance response curves from every month from October 2012 until May 2013 in ice (a), under-ice water (UIW, c) and the 0–3-m layer (e) at Krogarviken (A) and in ice (b), UIW (d) and the 0–15-m layer (f) at Storfjärden (B). Note different scale for y axis in UIW at Krogarviken (A) (I).

that the initial community in the open water is a combination of sea ice algae and pelagic species, and that the pelagic community after ice break-up is influenced by the pelagic species rather than the seeding effect from the sea ice as earlier suggested (Mikkelsen et al. 2008, Riaux-Gobin et al. 2011).

The photosynthetic activity in the water column, based on results of incubation under various light intensities, decreased during the fall (Fig. 7e,f). In October the photosynthetic activity was less than 5 mg C m^{-3} h^{-1} at site A and less than 10 mg C m^{-3} h^{-1} at site B. Biomass increased during January–March (Fig. 5 c–f), but the photosynthetic activity in the water column and the UIW was close to zero, indicating that the community was mainly hetero- and/or mixotrophic. In April before ice break-up the photosynthetic activity at site A in the UIW was close to 40 mg C m^{-3} h^{-1} (Fig. 7c), suggesting that the dinoflagellates, which dominated the UIW community, were autotrophic. Similarly, the photosynthetic activity in the water column just before ice break-up was higher compared to other sampling occasions. During this time dinoflagellates, probably the autotrophic ones, also dominated the water column. The photosynthetic activity remained high right after ice break-up, but despite the increasing biomass the photosynthetic activity decreased during the spring bloom at both study sites (Fig. 7e,f). Wang et al. (2014) observed a similar decrease in photosynthetic activity during the spring bloom, which was related to community change from the diatom dominance to dinoflagellate dominance. However, a similar change in community composition was not observed in the succession study (I), and results may suggest that the community was dying and/or changing from an autotrophic community to a mixo- and/or heterotrophic community.

5.3. Natural succession of sea-ice algal communities

5.3.1 Natural succession throughout the ice-covered season

The sea-ice community was significantly different compared to the water column communities based on the dendrogram produced from the Bray-Curtis similarity coefficients of both OTUs and the microalgal biomass (see section 5.2). The results agree with the previous work by Tuschling et al. (2000) and Majaneva et al. (2012), and indicate that the sea-ice community forms a unique community compared to the water column communities throughout the cold-water season. In the succession study (I) the sea ice microalgae could have originated from the benthic community, especially at the shallow site A. Based on the OTU results, the species richness in the water column decreased at the beginning of the ice-covered season compared to the fall, and increased later during the ice-covered season, indicating that algal succession continued throughout the ice-covered season (I). The dominant species (diatoms *Pauliella taeniata*/*Navicula* sp., *Nitzschia frigida*, *Chaetoceros* spp. and *Melosira* spp., dinoflagellates including *Scrippsiella* complex and *Heterocapsa arctica* sub. *frigida* and green algae *Klebsormidium flaccidum*) in the spring ice were also present in the young ice, indicating that the spring ice community was originally developed from the new ice community.

The sea-ice algal succession at site A began with a low biomass, and the community was dominated by flagellates (Fig. 4 and 5a). This period was followed by a bloom phase with high biomass. Similar to previous studies in the Baltic Sea, the spring ice community was dominated by diatoms and dinoflagellates

(Haecky et al. 1998, Haecky and Andersson 1999, Meiners et al. 2002, Piiparinen et al. 2010, Rintala et al. 2010). The microalgal succession at site A resembled the succession of oceanic sea ice, where the succession begins with a pre-bloom with low biomass and flagellate domination (cf. Leu et al. 2015). The pre-bloom in the oceanic ice is followed by the bloom phase, where biomass increases and diatoms are the most dominant group in the ice. At site B, where the sea ice broke up in the middle of the ice-covered season, the succession did not follow the same pattern as at site A or that described by Leu et al. (2015). The highest microalgal biomass (290 mg C m^{-3}) occurred right after the reformation of the sea-ice cover and decreased towards ice break-up (Fig. 4). It is possible that the ice for the newly formed ice-cover was originally formed in another location at site B. A decrease in microalgal biomass was observed before the final melt and ice break-up, indicating brine channel widening due to the increase in ice temperature and release of the brine due to gravity drainage. The last phase of the sea ice algae succession is the post-bloom, during which the microalgal biomass decreases and the community changes from a diatom-dominated community to a heterotrophic community (Leu et al. 2015). The post-bloom was not detected in 2013. However, the post-bloom phase in the Baltic Sea ice has been observed and described earlier by Kaartokallio (2004).

In January the photosynthetic activity in the ice was similar compared to the photosynthetic activity in the water column indicating that the community was mainly hetero- and/or mixotrophic. Measured from the entire ice core, photosynthetic activity in the ice was highest at both sites at the end of February. The photosynthetic activity in the ice was less than 10 mg C m^{-3} h^{-1} (Fig. 7a,b), and comparable to the October situation in the water column.

Based on the dendrogram results of the microalgal biomasses the community composition in the sea ice resembled more the spring bloom community composition than the fall or winter water column community compositions (Fig. 6b), suggesting the possible seeding effect from the sea ice. Whether sea ice algae seed the spring bloom or not is a highly debated subject. Results from the succession study (I) support the earlier observation, that sea ice algae partly contribute to water column production during ice melt (Michel et al. 1993). The release time of sea ice algae explains their later role in pelagic production, and e.g. pelagic grazers consume sea ice algae released earlier during the ice-covered season (Michel et al. 1993). Nevertheless, the contribution of sea ice algae to the pelagic production was relatively low, which could also be related to the tendency of some ice algae species to form large, non-symmetric colonies, with high sinking velocity, which results in their sedimentation after release from the sea ice (Padisák et al. 2003).

5.3.2 Natural succession of the spring ice in various ice layers

On February 28th 2011, i.e. the beginning of the three-week experimental study (II), the total biomass in the untreated ice with snow cover (UNT) was distributed evenly throughout the sea ice, and diatoms and dinoflagellates typical of the late season community in the Baltic Sea ice were the most dominant groups (Norrman and Andersson 1994; Haecky and Andersson 1999; Rintala et al. 2006; Kaartokallio et al. 2007; Piiparinen and Kuosa 2011). Diatoms were dominated by *Pauliella*/*Navicula* and dinoflagellates by unidentified peridinioid dinoflagellates and *Heterocapsa arctica* subs. *frigida*. Small (< 10-µm) unidentified flagellates were the third most abundant microalgal group alongside diatoms and

dinoflagellates, but despite their high cell numbers, the contribution of small flagellates to total microalgal biomass remained low. During the experiment microalgal biomass increased in all ice layers, but the vertical distribution of biomass was not even between the ice layers (Fig. 8). In addition, the dominance of different species varied between the ice layers and green algae replaced the flagellates as the third most abundant microalgal group. Particularly the biomass of diatoms, dominated by *Nitzschia frigida*, increased in the top 5-cm layer, but total biomass was still the lowest compared to the layers below the top 5-cm layer. The biomass of diatoms, dominated by *Pauliella*/*Navicula*, also increased between March 7th and 14th 2011 in the 5- to 10-cm layer. In the middle layer the biomass of diatoms increased especially between February 28th and March 7th, and in addition to *Pauliella*/*Navicula*, *Nitzschia frigida* and genera *Chaetoceros*, *Thalassiosira* and *Melosira* became abundant. Biomass was highest in the bottom 10-cm layer, and in addition to *Pauliella*/*Navicula*, *Nitzschia frigida* dominated the diatom community. The bottom 10-cm layer was also characterized by an increase in cyanobacteria.

Green algae are not generally abundant in Baltic Sea ice (Norrman and Andersson 1994, Haecky and Andersson 1999, Meiners et al. 2002, Kaartokallio et al. 2007, Rintala et al. 2010), but they are very typical and one of the dominant algal groups of total biomass alongside diatoms and dinoflagellates in the sea ice in front of Tvärminne Zoological Station (I–III, Piiparinen and Kuosa 2011). The mean depth of this area is 3 m and the benthic species may contribute the sea-ice microalgae community. Two of the most dominant green algae species are *Chlamydomonas caudata* and *Klebsormidium flaccidum*, which is a filamentous charophyte green algae found on the shoreline rocks (Rindi and Guiry 2004). This species was also reported in Baltic Sea ice in the late 1920s, with its previous name *Hormidium flaccidum* (Häyren 1929). In this study (II) these two species (*C. caudata* and *K. flaccidum*) were abundant especially in the 5- to 10-cm and the middle layers, probably due to the optimal light environment at this depth, because chlorophytes generally grow better under higher irradiance (Richardson et al. 1983). In both layers the biomass of green algae multiplied between February 28th and March 14th 2011 (II).

Photosynthetic activity was measured as part of the UVR experiment in 2011 at site A, and it varied between the different ice layers during the study (Fig. 9). On February 28th, at the beginning of the study, no clear differences were observed in the photosynthetic activity between the layers (Fig. 9a,d,g,j, UNT treatment). On day 7 (Mar 7th), the differences between the ice layers became clearer and the highest photosynthetic activity (0.70 mg C [mg chl *a*]$^{-1}$ h^{-1}) was observed in the middle layer, while photosynthetic activity was lower in the top 10-cm layer and the bottom layer (0.46–0.49 mg C [mg chl *a*]$^{-1}$ h^{-1}) (Fig. 9b,e,h,k, UNT-treatment). Similar results have been observed in previous studies from the Baltic Sea, in which bottom ice communities have been associated with lower photosynthetic activity than the surface communities (Piiparinen et al. 2010; Rintala et al. 2010; Piiparinen and Kuosa 2011). On day 14 (Mar 14th), photosynthetic activity increased in the middle layer, and the difference between the middle and the bottom 10-cm layer became clearer compared to the preceding weeks (Fig. 9c,f,i,l, UNT treatment). Photoinhibition was observed in all ice layers (Fig. 9a–l) due to high light intensities (>2000 µmol m^{-2} s^{-1}) used in the measurements of photosynthesis-irradiance (see section 4.2.4.), but it was the strongest in the bottom 10-cm layer, indicating shade adaptation of the bottom ice community.

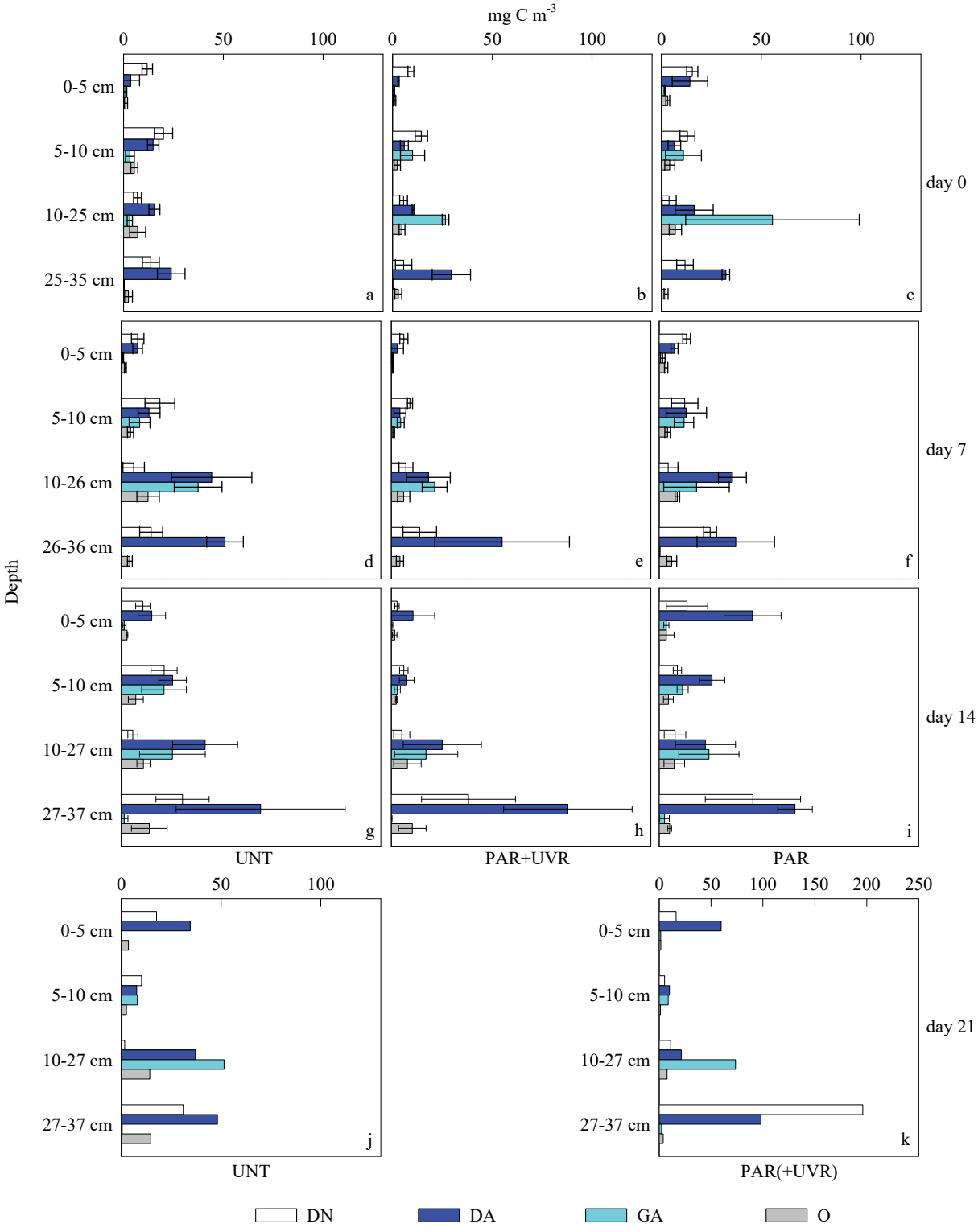

Fig. 8. Biomasses (mean ± sd, mg C m^{-3}) of dinoflagellates (Dinophyceae, DN), diatoms (Diatomophyceae, DA), green algae (GA) and others (O) in the treatments with natural snow cover (UNT), exposed to the full solar spectrum (PAR+UVR and PAR(+UVR)) and covered with UV filter foil (PAR) in each ice layer at each sampling in the UVR-experiment. Note different scale for x axis in the PAR(+UVR) treatment on day 21 (II, III).

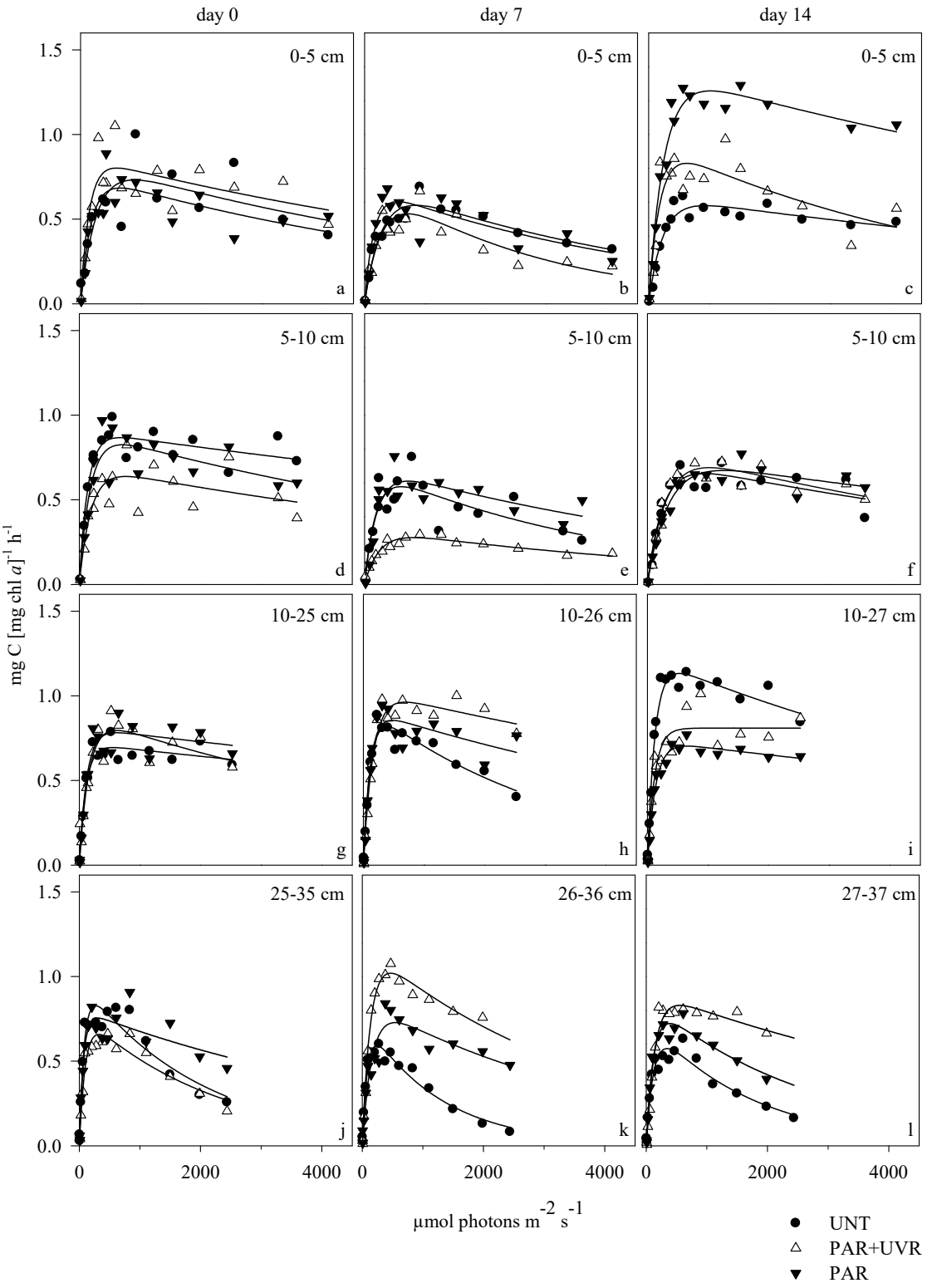

Fig. 9. Photosynthesis-irradiance response curves in the treatments with natural snow cover (UNT), exposed to the full solar spectrum (PAR+UVR) and covered with UV filter foil (PAR) in each ice layer at each sampling in the UVR-experiment (II).

5.4. Effect of enhanced solar irradiance on sea ice algae

5.4.1 Responses in species-specific microalgal biomass and photosynthetic activity

The average daily irradiance during the first week (Feb 28th–Mar 7th) of the experiment in 2011 varied from 52 to 176 W m^{-2} and the UVR from 8 to 22 W m^{-2} (II, III). During the second week of the experiment (Mar 7th–Mar 14th) the irradiance varied from 52 to 250 W m^{-2} and the UVR from 8 to 30 W m^{-2}. The PAR+UVR treatment was exposed to this level of irradiance, whereas the sea ice in the PAR treatment was exposed to approximately 82 % of this irradiance > 390 nm (see section 4.1.2.). Ice in the UNT treatment was only exposed to a fraction of this solar irradiance, as the 10-cm thick snow layer under melting conditions, such as in this study, typically reduces visible transmittance to < 5 % (Perovich 2007). For UVR, a 5-cm thick snow layer reduces 90 % or more of the incident UVR based on model calculations (Perovich 1993). The irradiance values (up to 250 W m^{-2}) were higher compared to a study performed in the same area on March 2nd–March 23rd 2005 (up to 200 W m^{-2}; Piiparinen and Kuosa 2011). In 2005 the irradiance doubled (from 100 to 200 W m^{-2}) during the experiment, but a similar doubling was not seen in 2011, due to increased cloudiness during the second week of the experiment.

The ice layers showed differences in microalgal biomass between the various light manipulation treatments (Fig. 8). Therefore it is important to study the effects of light on all layers of the sea ice. The irradiance begins attenuating already in the top 5-cm layer of the ice (Müller et al. 2016), and thus the amount of irradiance that reaches the bottom layer, especially if the sea ice is thick, is clearly less than in the surface layer. In the UVR experiment, the lowest microalgal biomass and the highest variation between the treatments was observed in the top 10-cm layer (Fig. 8), but due to the high patchiness throughout the experimental field, a significant difference between the treatments was only found in the top 5-cm layer on day 7 (Latin square ANOVA $P < 0.05$). The biomass of various microalgal groups did not differ significantly between the treatments in any layer during the experiment (Latin square ANOVA $P > 0.05$), but some differences were found at the species level, indicating that the increased amount of solar irradiance was harmful for some species.

Sea-ice communities are exposed to increased amounts of solar irradiance (PAR and UVR) when a thin snow layer covers the ice, and the PAR+UVR treatment represented such a situation in this experiment. The biomass of *Heterocapsa arctica* ssp. *frigida* was significantly higher (Latin square ANOVA $P < 0.05$, Tukey's b $P < 0.05$) in the UNT treatment than in the PAR+UVR and PAR treatments in the 5- to 10-cm layer on day 14, but not in the top 5-cm layer (Latin square ANOVA $P > 0.05$, Tukey's b $P > 0.05$). The UVR-sensitivity of *H. arctica* ssp. *frigida* may have been masked by the effect of PAR in the PAR+UVR treatment, as the species did not benefit from higher light intensities in the top 10-cm layer in the PAR treatment. The biomass of < 20-µm unidentified flagellates, which also included unidentified dinoflagellates, decreased in all ice layers. The similar effect of enhanced PAR on dinoflagellates and < 20-µm unidentified flagellates was observed in the same area by Piiparinen and Kuosa (2011).

Certain taxa belonging to various microalgal groups, benefited from enhanced PAR and UVR exclusion. The biomass of the *Scrippsiella* complex, which consisted of pigmented dinoflagellates, mainly *Scrippsiella hangoei*, *Biecheleria baltica* and *Gymnodinium corollarium*, was significantly lower in the PAR+UVR treatment than in the UNT and PAR treatments in the top 5-cm layer on day 14 (Scheirer-Ray-Hare $P = 0.05$, Mann Whitney U $P = 0.05$). An even more notable response of UVR exclusion was observed in diatoms. High diatom biomass accumulation was observed on day 14 in the top 5-cm layer in the PAR treatment, where the pennate diatom *Nitzschia frigida* occurred as large colonies with significantly higher biomass than in the PAR+UVR treatment (Latin square ANOVA $P < 0.01$, Tukey's b $P < 0.05$). This result further emphasizes the UVR sensitivity of *Nitzschia* sp. observed in previous studies, in which exposure to UVR decreased the amount of light-harvesting pigments of *N. closterium* (synonym *Ceratoneis closterium*) (Buma et al. 1996), the gliding motility of *N. linearis* (Moroz et al. 1999) and the photosynthetic rate of *N. palea* and other *Nitzschia* (Arts and Rai 1997, Nilawati et al. 1997, Wulff et al. 2000) and lead to a loss of *N. longissima* (Santas et al. 1998). However, certain benthic pennate diatom communities and assemblages have been observed to tolerate UVR (Wulff et al. 2008a,b, Karsten et al. 2009). The accumulation of pennate diatom species in the lower ice layer and their complete absence in the surface layer was observed by Piiparinen et al. (2010) in the sea ice of the Bothnian Bay, and only the centric diatom *Chaetoceros wighamii* survived under UVA exposure. The decreasing biomass of pennate diatoms under UVR exposure is explained with the typical 2-chloroplast configuration, which is proposed not to provide shelter from the UVR exposure compared to the multi-chloroplast configuration of centric diatoms (Karentz et al. 1991a).

Green algae (mostly *Chlamydomonas caudata* and *Klebsormidium flaccidum*) were present in all ice layers, especially in the 5- to 10-cm and middle layers of the ice (Fig. 8; II). The biomass of green algae in the top 5-cm layer was small in all treatments, indicating that green algae may not have benefited from high PAR. This did not correspond to the results of previous studies, where various dominant green algae species have grown better under higher irradiance (Richardson et al. 1983, Piiparinen and Kuosa 2011), suggesting that all green algae do not benefit from high PAR. On day 14, the biomass of green algae in the 5- to 10-cm layer was significantly higher (Scheirer-Ray-Hare $P < 0.05$, Mann Whitney U $P < 0.05$ and $P = 0.05$, respectively) in the UNT and PAR treatments than in the PAR+UVR treatment, indicating a negative response to UVR. The same effect of UVR was not observed below the 10-cm layer, which suggests that the effect of UVR does not penetrate to the deeper layers. Under natural conditions UVR enters the sea ice, and thus for example the accumulation of diatom *N. frigida* in the top 5-cm layer, described in the previous chapter, is very unlikely to happen in natural situations in the Baltic Sea. However, changes in community composition are possible if the sea-ice community is exposed to higher UVR levels due to thinner sea-ice cover and possible ozone depletion.

Photosynthetic activity (PI curves) varied between the treatments in the UVR experiment. On day 7 (Mar 7th), UVR exposure decreased photosynthetic activity in the top 10-cm layer of the ice in the PAR+UVR treatment compared to the UNT

and PAR treatments (Fig. 9b,e), suggesting the harmful effect of UVR especially in the top 10-cm layer of the ice. However, below the 10-cm layer the photosynthetic activity was highest in the PAR+UVR treatment and lowest in the UNT treatment (Fig. 9h,k). The low microalgal biomass and decreased self-shading by microalgae in the upper layers of the PAR+UVR treatment likely resulted in increased amounts of photosynthetic active radiation in the deeper layers. On day 14 (Mar 14th) the effect of UVR exposition was lower in the 5- to 10-cm layer and the photosynthetic activity was similar in all treatments (Fig. 9f), suggesting that the microalgae community at the depth of 5 to 10 cm was able to recover after 14 days of UVR exposure. However, in the top 5-cm of the ice layer the photosynthetic activity in the PAR treatment was 1.6-fold higher compared to the PAR+UVR treatment (Fig. 9c). The high photosynthetic activity in the top 5-cm layer of the PAR treatment reflected the dominance of *Nitzschia frigida*. In the middle layer and bottom 10-cm layer the highest photosynthetic activity was in the UNT and PAR+UVR treatments, respectively, suggesting the increased self-shading by microalgae in the PAR treatment (Fig. 9i,l).

5.4.2 Responses to MAA concentration

At the beginning of the experiment on February 28th 2011, the average MAA concentration throughout the experimental field was highest in the top 5-cm layer, but MAA concentration showed high patchiness within the experimental field (mean 1.79 ± SD 1.16 μg L^{-1}) (Fig. 10). In the 5- to 10-cm layer, the concentration of MAAs decreased to approximately one third of the concentration observed in the top 5-cm layer (mean 0.67 ± SD 0.38 μg L^{-1}). Below the top 10-cm layer the concentration of MAAs was even lower (0.05 ± SD 0.02 μg L^{-1}), which verified the rapid UVR attenuation. The concentration in the top 5-cm layer was similar to the previous MAA concentration reported from the surface (top 12-cm) layer (mean 1.37 ± 0.39 μg L^{-1}) from the same area at the same time in the spring (Uusikivi et al. 2010). Below the 10-cm layer the MAA concentration was one third of the concentration observed in an earlier study performed at the same site, indicating that the species composition in the ice varied, but milder winter in the earlier study also resulted in the variation of MAA concentrations between the studies (Uusikivi et al. 2010). The MAA pool was dominated by shinorine and palythine, and in addition, low concentrations (0.02 μg L^{-1}) of an unknown compound (absorption max 333 nm) were observed in the top 5-cm layer. The strong decline in palythine concentration with depth indicates that palythine synthesis is triggered by shorter wavelengths (absorption max 320 nm) than the synthesis of shinorine (absorption max 334 nm) or the unknown compound (absorption max 333 nm), and thus the spectral irradiance affects the contents of the MAA pool between various ice layers.

During the first two weeks of the experiment (Feb 28th–Mar 14th 2011), the concentration of MAAs remained within the range measured on day 0, but changes between the treatments were observed in the MAA:chl *a* ratios and contents of the MAA pool (Fig. 10). After two weeks of exposure to full solar irradiance (without the snow cover or UV filter film), the MAA:chl *a* ratio in the PAR+UVR treatment increased especially in the 5- to 10-cm layer. However, the PAR+UVR treatment differed significantly from the other treatments only

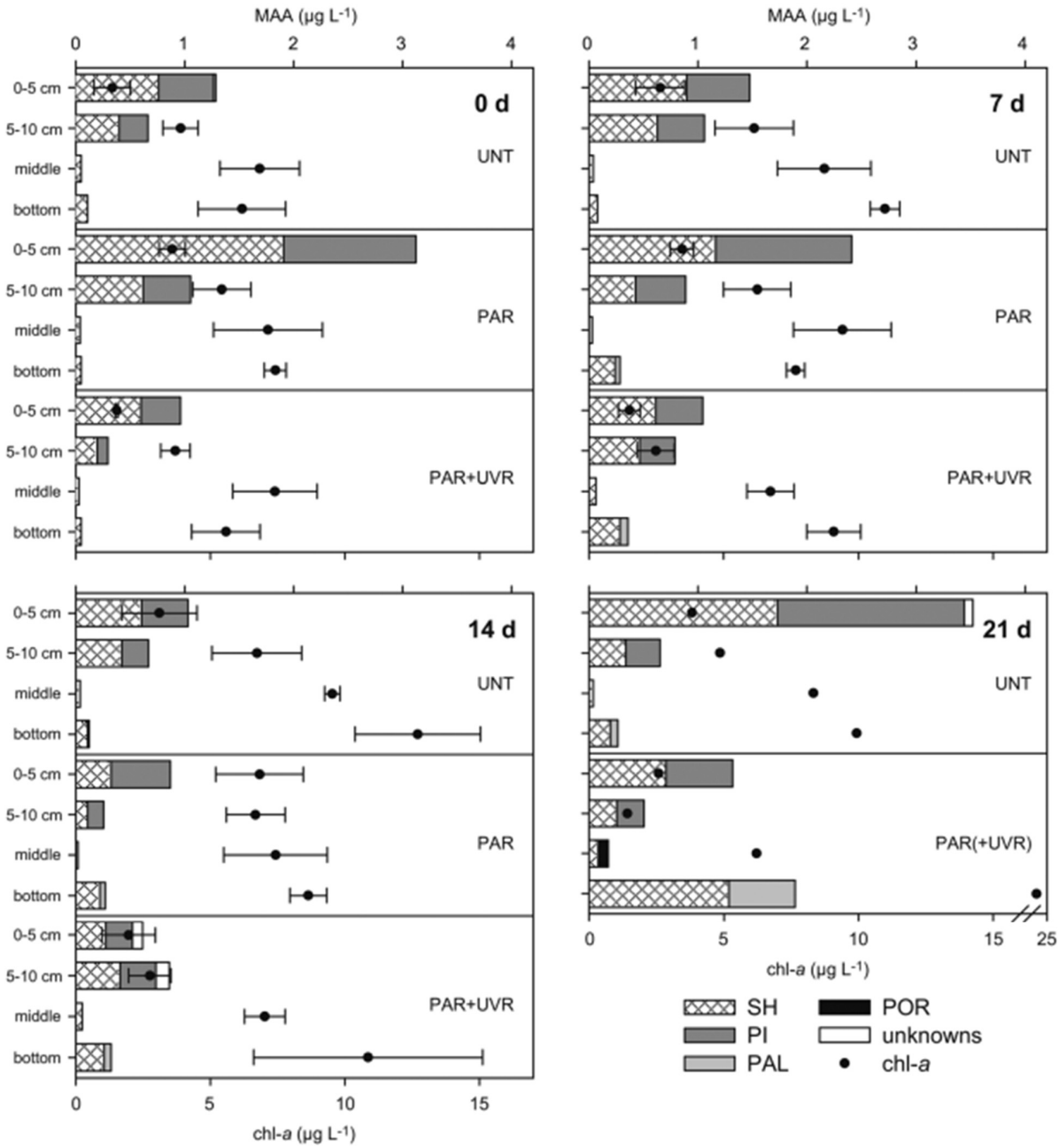

Fig. 10. Concentration of mycosporine-like amino acids (shinorine, SH; palythine, PI; palythinol, PAL; porphyra, POR and unknowns) (µg L^{-1}) and mean chlorophyll *a* (µg chl a L^{-1}) and standard deviations except for the day 21 in the treatments with natural snow cover (UNT), exposed to the natural solar spectrum (PAR+UVR), covered with UV filter foil (PAR) and re-exposure to the natural solar spectrum (PAR(+UVR)) in each ice layer at each sampling in the UVR-experiment (III).

in the 5- to 10-cm layer on day 7 ($P < 0.05$, df = 8). The MAA pool was dominated by shinorine, palythine and a compound with the absorbance characteristics of the M335/360 complex, which is a condensation product of shinorine and palythine. In the top 10-cm layer of the PAR treatment, the UVR exclusion resulted in a decrease of the MAA:chl *a* ratio. In addition, the shinorine concentration decreased in the top 10-cm layer of the PAR treatment, and palythine became the dominant MAA. These results indicate that sea ice algae respond to changes in the quantity and quality of light by changing their MAA concentration and content of the MAA pool. The more diverse MAA pool under UVR exposition may result in greater protection against UVR-induced stress. In addition, variation in MAA pool content is explained with taxonomic affiliation. Karentz et al. (1991b) have shown that amounts of individual MAAs relative to the total MAA content varied between different species. However, in the sea ice, especially in the top layer of the ice, change in the community composition through the introduction of new species into the community is limited, as communities are isolated from the UIW. Thus the variation is related to changes in the biomass and MAA contents of a few dominant species rather than the introduction of new species into the surface community.

During the third week of the experiment (Mar 14th–Mar 21st) the increase in weekly irradiance resulted in an increase of MAA synthesis due to both the PAR and UVR regions of the spectrum. In the UNT treatment the concentration of MAA increased more than 2-fold in the top 5-cm layer, but the chl *a* concentration decreased in the upper layers compared to day 14, which resulted in an increasing MAA:chl *a* ratio. However, as snow cover affected the quantity and quality of light entering the ice, the inductive wavelengths were mostly in the PAR region. Shinorine and palythine were the dominant MAAs, but a small concentration of unknown compound (absorption max 320 nm) was also present in the top 5-cm layer. In the PAR treatment the UV filter film was removed before the third week of the experiment, exposing the ice again to the incident solar radiation, including UVR, which led to a 2.7-fold overall increase in the MAA concentration. The production of MAAs in the top 5-cm layer during the last week of the experiment was concurrent with the pennate diatom *Pauliella taeniata*, the biomass of which increased in both the untreated ice and the PAR(+UVR) treatment. However, as MAAs are quite stable and can be preserved in a freezer (see section 4.2.5), implying that MAAs can be preserved also in the sea-ice environment, the origin of the MAAs may be from another microalgal group, although they did not dominated the community during the sampling. Elliot et al. (2015) did not observe a significant correlation between MAAs and diatoms or dinoflagellates, but a significant correlation was observed between MAAs and various flagellate taxa in the water column.

In addition, the concentration of 334-porphyra increased in the middle layer of the ice during the third week of the experiment, and in the bottom layer the palythinol concentration increased more than 10-fold in the PAR(+UVR) treatment. The increase in 334-porphyra in the middle layer of the ice coincided with an increase in *Klebsormidium flaccidum*, which suggests that 334-porphyra was synthesized by this species. MAAs in the genus *Klebsormidium* have been studied in terrestrial habitats for the species *Klebsormidium fluitans*, which synthesizes an unidentified MAA with an

absorption maximum at 324 nm (Kitzing et al. 2014, Holzinger and Pichrtová 2016). However, the linkage between *Klebsormidium flaccidum* and 334-porphyra needs further studied. The increased concentration of palythinol (absorption max 332 nm) in the bottom layer of the ice suggested that for certain species MAA synthesis is a response to increased intensities of visible light and not only to UVR. The increased concentration of MAAs in the bottom layer was related to a 2.5-fold increase in the chl *a* concentration and microalgal biomass during the third week of the experiment (III).

6. CONCLUSIONS

The cold-water season in the Baltic Sea is highly dynamic with various algal communities present in the sea ice and the water column. Based on community composition, the general succession pattern of microalgae during the cold-water season could be divided into five distinct groups. These groups represented fall, winter, UIW and spring, and the sea ice was additionally separated from the water microalgal column communities. The overarching succession in the water column is from a flagellate-dominated community in fall/winter towards a diatom- and dinoflagellate-dominated community in the spring. In addition, a UIW bloom occurs before ice break-up, and is dominated by species that are not common in the ice.

Similarly to the succession of the microalgal community in the water column, the succession in the ice shifts from a flagellate-dominated community in the winter ice towards a diatom- and dinoflagellate-dominated community in the spring ice. However, various microalgal groups and species dominate in the different ice layers. At the early stage the sea-ice algal biomass is evenly distributed between the ice layers, but later the most pronounced biomass increase is detected in the bottom 10-cm layer of the 40 cm thick sea-ice cover.

Ultraviolet radiation is one of the controlling factors of microalgal communities in Baltic Sea ice. Increased availability of PAR together with UVR exclusion can cause an increase in both microalgal biomass and photosynthetic activity, and changes in community composition, especially in the surface layer of the ice. Sea ice algae respond to UVR exposure by increasing their concentration of MAAs and by producing a

variety of MAA compounds for widening their absorption range in UV wavelengths.

The microalgae community composition in the ice differs from the spring bloom community of the water column, and the initial open-water community is a combination of sea ice microalgae and pelagic species. This indicates that the pelagic community after ice break-up is influenced by pelagic species and sea ice microalgae do not have a large seeding effect on the spring bloom. Although the sea-ice algae community does not appear to contribute greatly to the water column phytoplankton growth and spring bloom after ice melt, sea ice algae contribute to the total PP during the ice-covered season and a high biomass of sea ice algae is released to the water column during the melting, which may serve as a food source for secondary consumers in the water column before and after ice melt.

These findings are important for understanding more in depth the microalgal succession during the cold-water season and how the water column and sea ice algae communities are linked. In the face of a warming climate and potentially large changes in winter severity between the winters, e.g. decreasing sea-ice extent and thickness of ice cover, these findings help us better understand and predict future changes in phytoplankton communities during the cold-water season and early summer.

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